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FIG. 1A

2H7scFv-Ig cDNA and predicted amino acid sequence:

HindIII	NcoI	2H7 V _L Leader Peptide→
~~~~~	~~~~~	
M D F Q V Q I F S F L L I S A S		
1 AAGCTTGGCG CC ATGGATTTC TCAAGTGCAG ATTTCAGCT TCCTGCTAAT CAGTGCTTCA		

	2H7 V _L →
V I I A R G Q I V L S Q S P A I L S A S	
61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT	
P G E K V T M T C R A S S S V S Y M H W	
121 CCAGGGGAGA AGGTACAAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG	

	BamHI
Y Q Q K P G S S P K P W I Y A P S N L A	
181 TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTG ATGCCCATC CAACCTGGCT	
S G V P A R F S G S G S G T S Y S L T I	
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC	
S R V E A E D A A T Y Y C Q Q W S F N P	
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA	

(Gly ₄ Ser) ₃ Linker	
P T F G A G T K L E L K G G G G S G G G	
361 CCCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG CGGGTGGCTC GGGCGGTGGT	

	2H7 V _H →
G S G G G G S S Q A Y L Q Q S G A E L V	
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGT	
R P G A S V K M S C K A S G Y T F T S Y	
481 AGGCCTGGGG CCTCAGTGAA GATGTCTGC AAGGCTTCTG GCTACACATT TACCAAGTTAC	
N M H W V K Q T P R Q G L E W I G A I Y	
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT	
P G N G D T S Y N Q K F K G K A T L T V	
601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA	
D K S S S T A Y M Q L S S L T S E D S A	
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG	
V Y F C A R V V Y Y S N S Y W Y F D V W	
721 GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG	

**FIG. 1B**

BclI  
-----human IgG1 Fc domain →

G T G T T V T V S D Q E P K S C D K T H  
 781 GGCACAGGG A CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAACCTCAC

T C P P C P A P E L L G G P S V F L F P  
 841 ACATGCCAC CGTGCCCAGC ACCTGAACTC CTGGGGGAC CGTCAGTCTT CCTCTTCCCC

P K P K D T L M I S R T P E V T C V V V  
 901 CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTACACATG CGTGGTGGTG

D V S H E D P E V K F N W Y V D G V E V  
 961 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

H N A K T K P R E E Q Y N S T Y R V V S  
 1021 CATAATGCCA AGACAAAGCC GCAGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

V L T V L H Q D W L N G K E Y K C K V S  
 1081 GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

N K A L P A P I E K T I S K A K G Q P R  
 1141 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

E P Q V Y T L P P S R D E L T K N Q V S  
 1201 GAACCACAGG TGTACACCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAAGC

L T C L V K G F Y P S D I A V E W E S N  
 1261 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

G Q P E N N Y K T T P P V L D S D G S F  
 1321 GGGCAGCCGG AGAACAACTA CAAGACCACG CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

F L Y S K L T V D K S R W Q Q G N V F S  
 1381 TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGAA CGTCTTCTCA

C S V M H E A L H N H Y T Q K S L S L S  
 1441 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

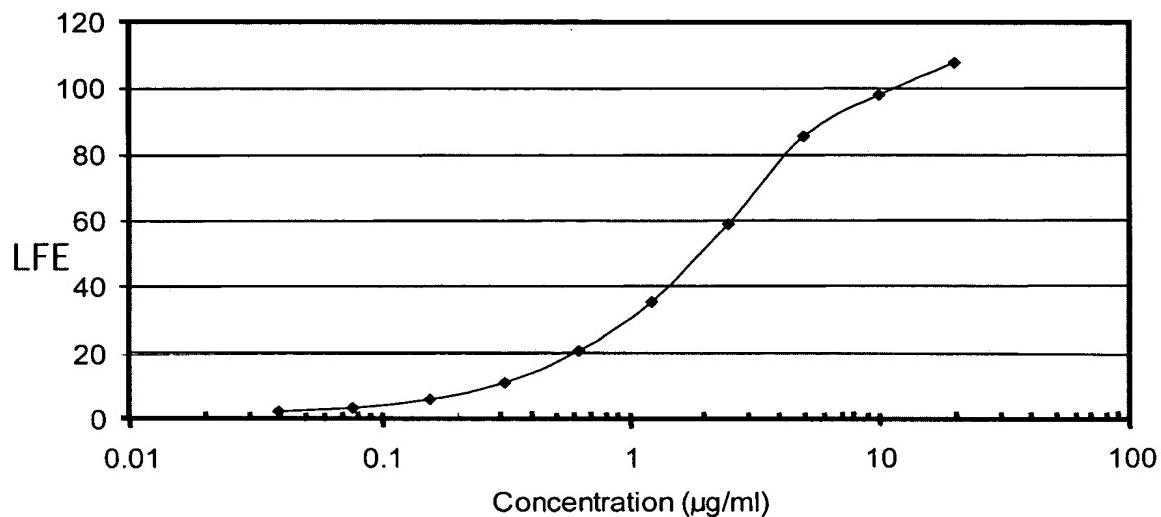
XbaI  
-----

P G K * S R  
 1501 CCGGGTAAAT GATCTAGA

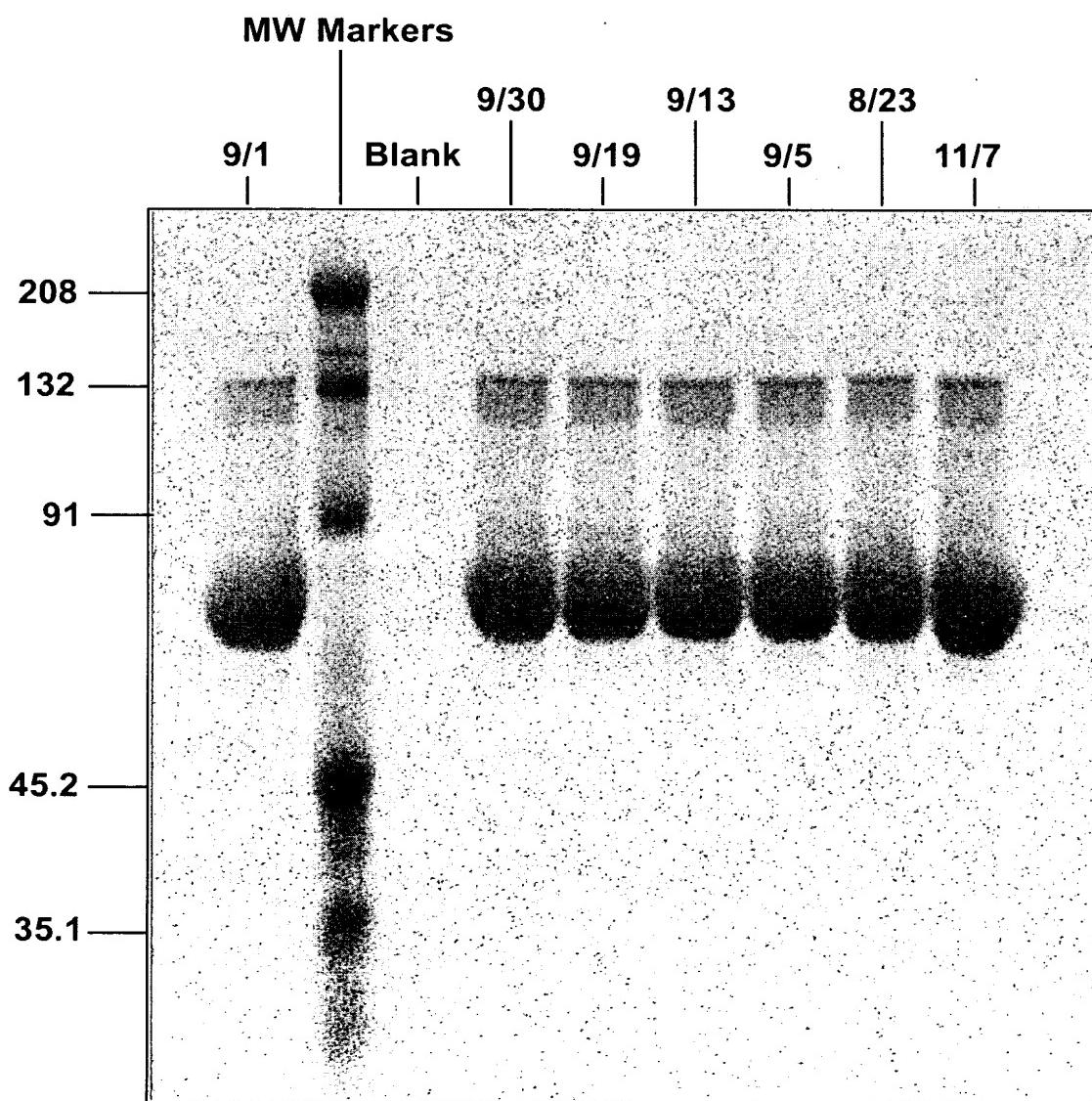
**FIG. 2**

Production Levels of 2H7 scFv (SSS-S)H WCH2 WCH3  
by Stable CHO Lines

2H7 scFv (SSS-S)H WCH2 WCH3 STANDARD CURVE



Clone	LFE @ 1:50 Estimated Concentration (mg/ml)
D2	26.156
IIIC6	25.755
IVA3	28.661
Spent bulk	29.664

**FIG.3****SDS-Page Analysis of  
2H7 scFv (SSS-S)H WCH2 WCH3 Protein**

**FIG. 4A**

**Complement Mediated B Cell Killing After Binding of CD20-targeted  
2H7 scFv (SSS-S)H WCH2 WCH3**

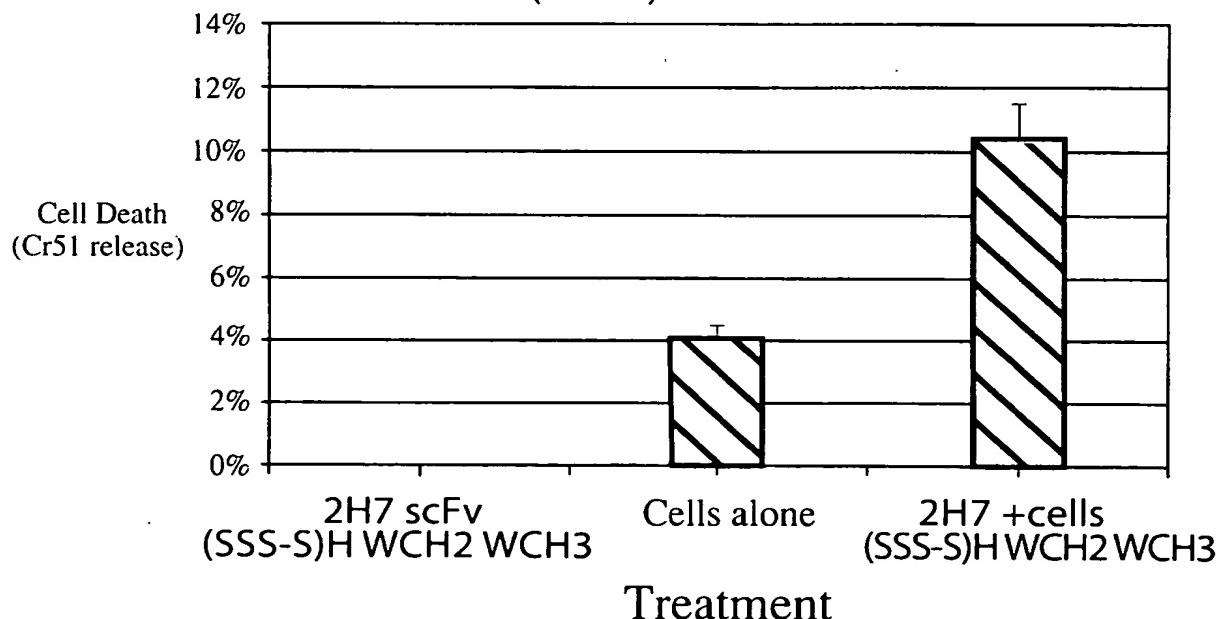
2H7scFv-Ig Concentration	RAMOS		BJAB	
	# LIVE CELLS / TOTAL CELLS		# LIVE CELLS / TOTAL CELLS	
20 µg/ml + complement	—	0.16	—	0.07
5 µg/ml + complement	—	0.2	—	N.D.
1.25 µg/ml + complement	—	0.32	—	0.1
Complement alone	—	0.98	—	0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

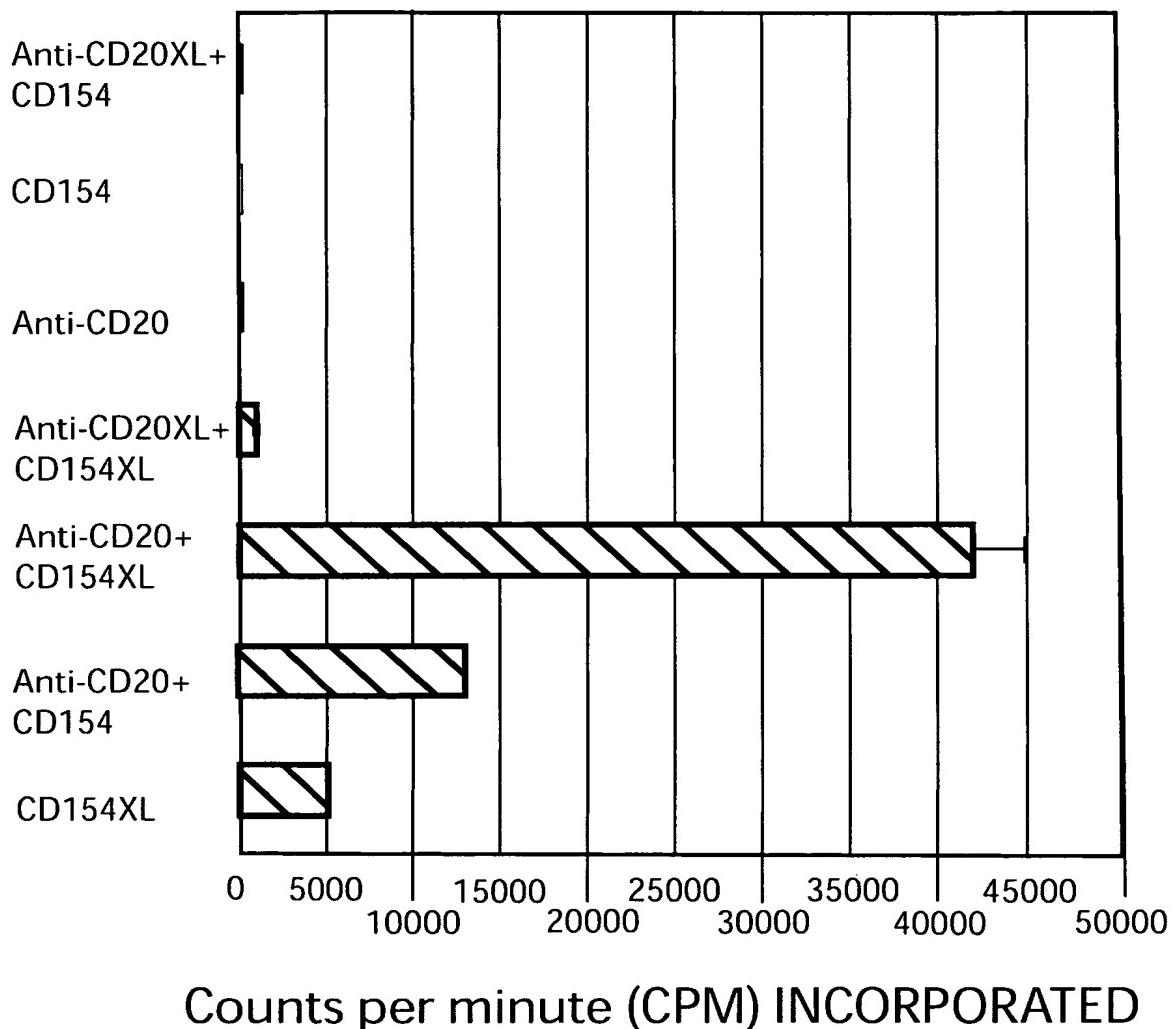
**FIG. 4B**

Antibody-dependent cellular cytotoxicity (ADCC) mediated by  
**2H7 scFv (SSS-S)H WCH2 WCH3**



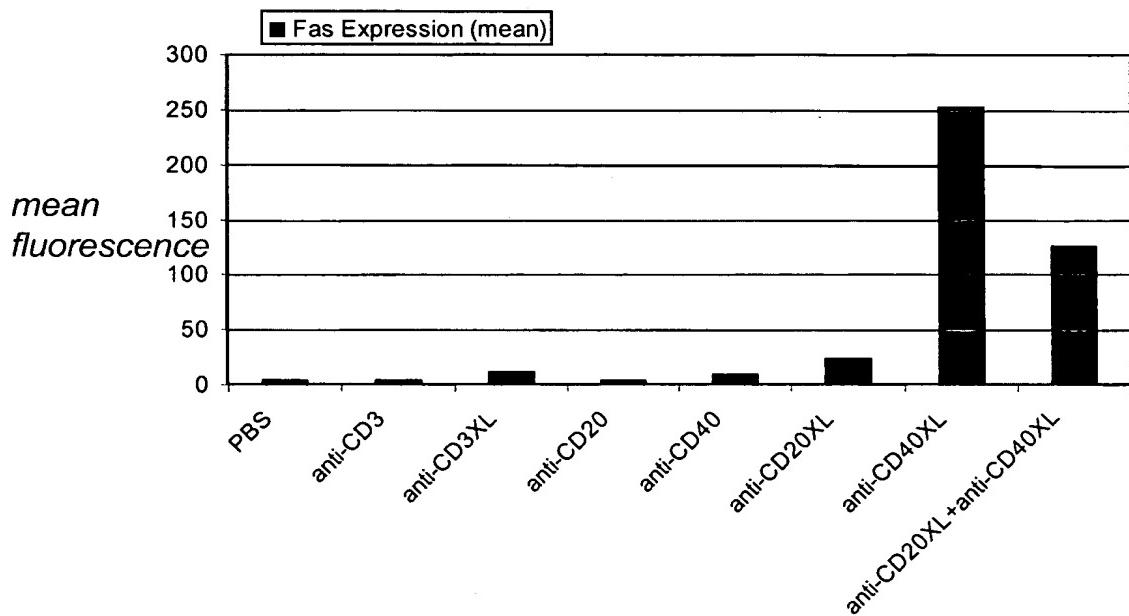
**FIG. 5**

Effects of Crosslinking of CD20 and CD40 Cell Surface Receptors on B Cell Proliferation:

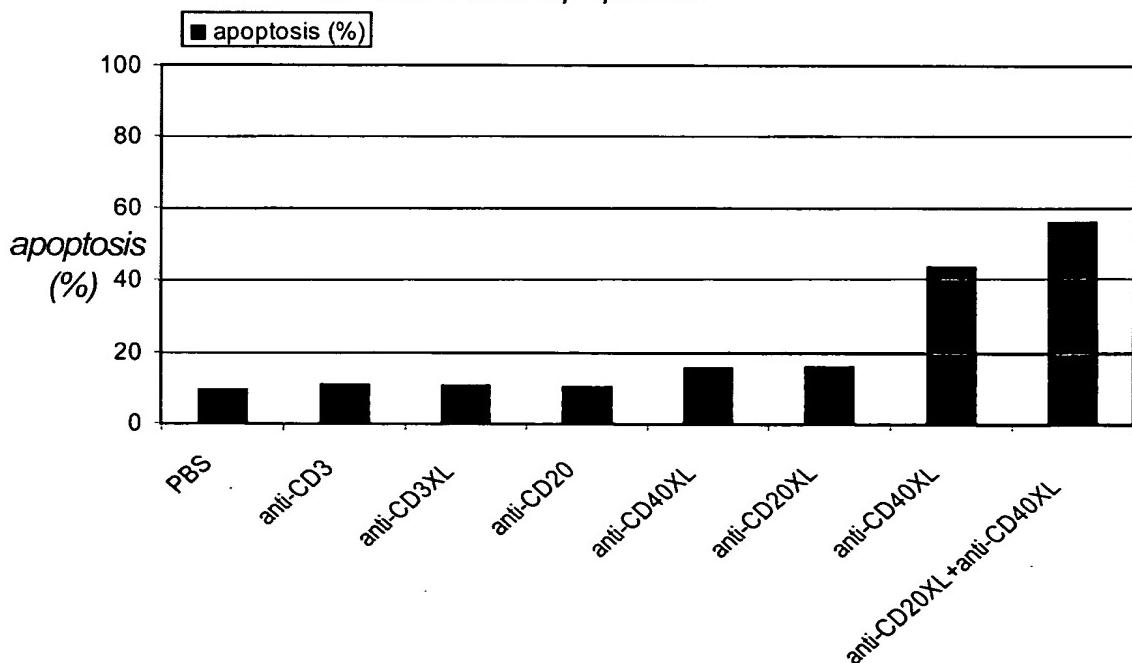


**FIG.6A**

*Effect of Simultaneous ligation of CD20 and CD40 on CD95 and apoptosis.*

**FIG.6B**

*Effect of Simultaneous ligation of CD20 and CD40 on CD95 and apoptosis.*



**FIG. 7A**

2H7-CD154 L2 cDNA and predicted amino acid sequence:

HindIII      NcoI      2H7 V _L Leader Peptide →	-----
	-----
1	M D F Q V Q I F S F L L I S A S
	AAGCTTGCCT CC ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA
2H7 V _L →	
61	V I I A R G Q I V L S Q S P A I L S A S
	GTCATAATTG CCAGAGGACA ATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT
121	P G E K V T M T C R A S S S V S Y M H W
	CCAGGGGAGA AGGTACAAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG
BamHI	
181	Y Q Q K P G S S P K P W I Y A P S N L A
	TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCATC CAACCTGGCT
241	S G V P A R F S G S G S G T S Y S L T I
	TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC
301	S R V E A E D A A T Y Y C Q Q W S F N P
	AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTAACCCA
(Gly ₄ Ser) ₃ Linker →	
361	P T F G A G T K L E L K G G G G S G G G
	CCCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT
2H7 V _H →	
421	G S G G G G S S Q A Y L Q Q S G A E L V
	GGATCTGGAG GAGGTGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG
481	R P G A S V K M S C K A S G Y T F T S Y
	AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAAGTTAC
541	N M H W V K Q T P R Q G L E W I G A I Y
	AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT
601	P G N G D T S Y N Q K F K G K A T L T V
	CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA
661	D K S S S T A Y M Q L S S L T S E D S A
	GACAAATCCT CGAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG
721	V Y F C A R V V Y Y S N S Y W Y F D V W
	GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

**FIG. 7B**

### human CD154/amino acid 48→

**site** Bcl/Bam hybrid

G T G T T V T V S D P R R L D K I E D E  
781 GGCACAGGGG CCA CGGT CAC CGT CTCTGAT CCA AGA AGGT TGG ACA AAG AT AGA AGA GTG AA

R N L H E D F V F M K T I Q R C N T G E  
841 AGGA ATCTTC ATGA AGATTT TGT ATT CATG AAA AC GATA C AGAG ATG CAA CAC AGG AGAA

R S L S L L N C E E I K S Q F E G F V K  
901 AGAT CCTTAT CCTT ACTGAA CTGT GAGG AG ATT AAA AGCC AGTT TGAGG CTTT GTGAA AG

BclI

D I M L N K E E T K K E N S F E M Q K G  
961 GATATA ATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTGAAAT GCA AAAAGGT

BclI

-----

D Q N P Q I A A H V I S E A S S K T T S  
1021 GATCAGAACATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

V L Q W A E K G Y Y T M S N N L V T L E  
1081 GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGTT AACCCCTGGAA

N G K Q L T V K R Q G L Y Y I Y A Q V T  
1141 AATGGGAAAC AGCTGACC GT TAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

-----

F C S N R E A S S Q A P F I A S L C L K  
1201 TTCTGTTCCA ATCGGGAAAGC TTCGAGTCAA GCTCCATTAA TAGCCAGCCT CTGCCTAAAG

S P G R F E R I L L R A A N T H S S A K  
1261 TCCCCCGGTA GATT CGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

P C G Q Q S I H L G G V F E L Q P G A S  
1321 CCTTGCGGGC AACAAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI

-----

V F V N V T D P S Q V S H G T G F T S F  
1381 GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XbaI

-----

G L L K L E * * S R  
1441 GGCTTACTCA AACTCGAGTG ATAATCTAGA

**FIG. 7C**

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

HindIII      NcoI  
 ----- 2H7 V_L Leader Peptide →  
 M D F Q V Q I F S F L L I S A S  
 1 AAGCTTGCCG CC ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 V_L →  
 V I I A R G Q I V L S Q S P A I L S A S  
 61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

P G E K V T M T C R A S S S V S Y M H W  
 121 CCAGGGGAGA AGGTCAACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI  
 -----  
 Y Q Q K P G S S P K P W I Y A P S N L A  
 181 TACCAAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTG ATGCCCATC CAACCTGGCT

S G V P A R F S G S G S G T S Y S L T I  
 241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAAATC

S R V E A E D A A T Y Y C Q Q W S F N P  
 301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly₄Ser)₃ Linker →  
 P T F G A G T K L E L K G G G G S G G G  
 361 CCCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V_H →  
 G S G G G G S S Q A Y L Q Q S G A E L V  
 421 GGATCTGGAG GAGGTGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG

R P G A S V K M S C K A S G Y T F T S Y  
 481 AGGCCTGGGG CCTCAAGTCAA GATGTCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

N M H W V K Q T P R Q G L E W I G A I Y  
 541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT

P G N G D T S Y N Q K F K G K A T L T V  
 601 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

D K S S S T A Y M Q L S S L T S E D S A  
 661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

V Y F C A R V V Y Y S N S Y W Y F D V W  
 721 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACCTTT ACTGGTACTT CGATGTCTGG

**FIG. 7D**

### human CD154/amino acid 108 →

BclI Bcl/Bam hybrid site

781 G T G T T V T V S D P E N S F E M Q K G  
GGCACAGGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTGAAAT GCAAAAGGT

BclII

-----

841 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGAAC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACAAACATCT

901 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCCCTGGAA

961 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

-----

1021 F C S N R E A S S Q A P F I A S L C L K  
TTCTGTTCCA ATCGGGAAAGC TTCGAGTCAA GCTCCATTAA TAGCCAGCCT CTGCCTAAAG

1081 S P G R F E R I L L R A A N T H S S A K  
TCCCCCGGTA GATTGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA

1141 P C G Q Q S I H L G G V F E L Q P G A S  
CCTTGCGGGC AACAAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG

NcoI

-----

1201 V F V N V T D P S Q V S H G T G F T S F  
GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

XbaI

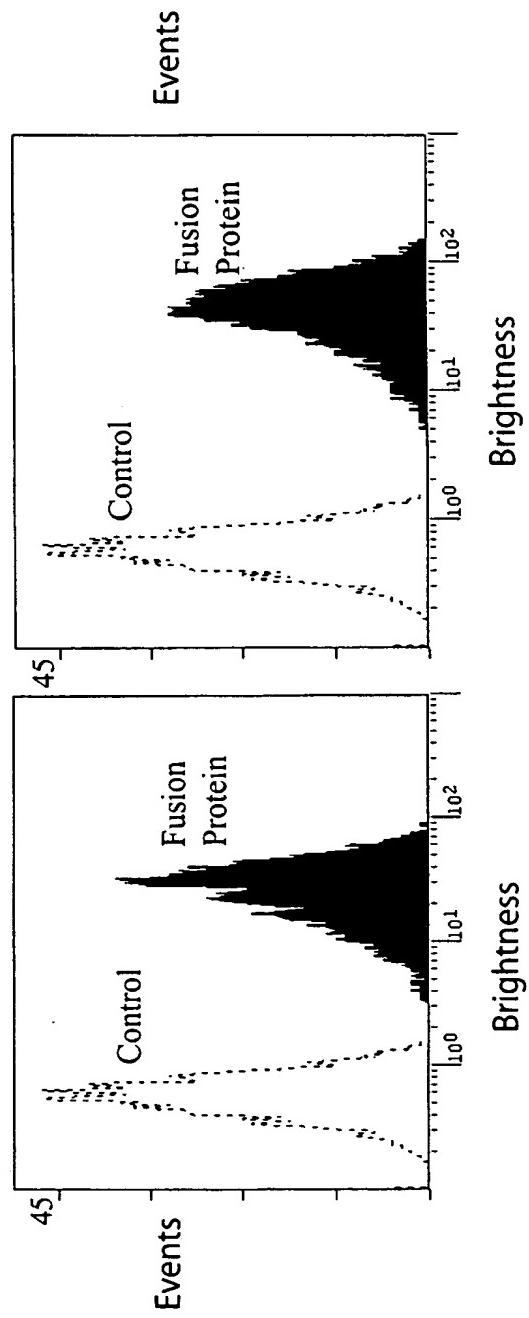
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1261 G L L K L E * * S R  
GGCTTACTCA AACTCGAGTG ATAATCTAGA

# FIG. 8

## Simultaneous Binding of 2H7scFv-CD154 Fusion Proteins to CD20 and CD40

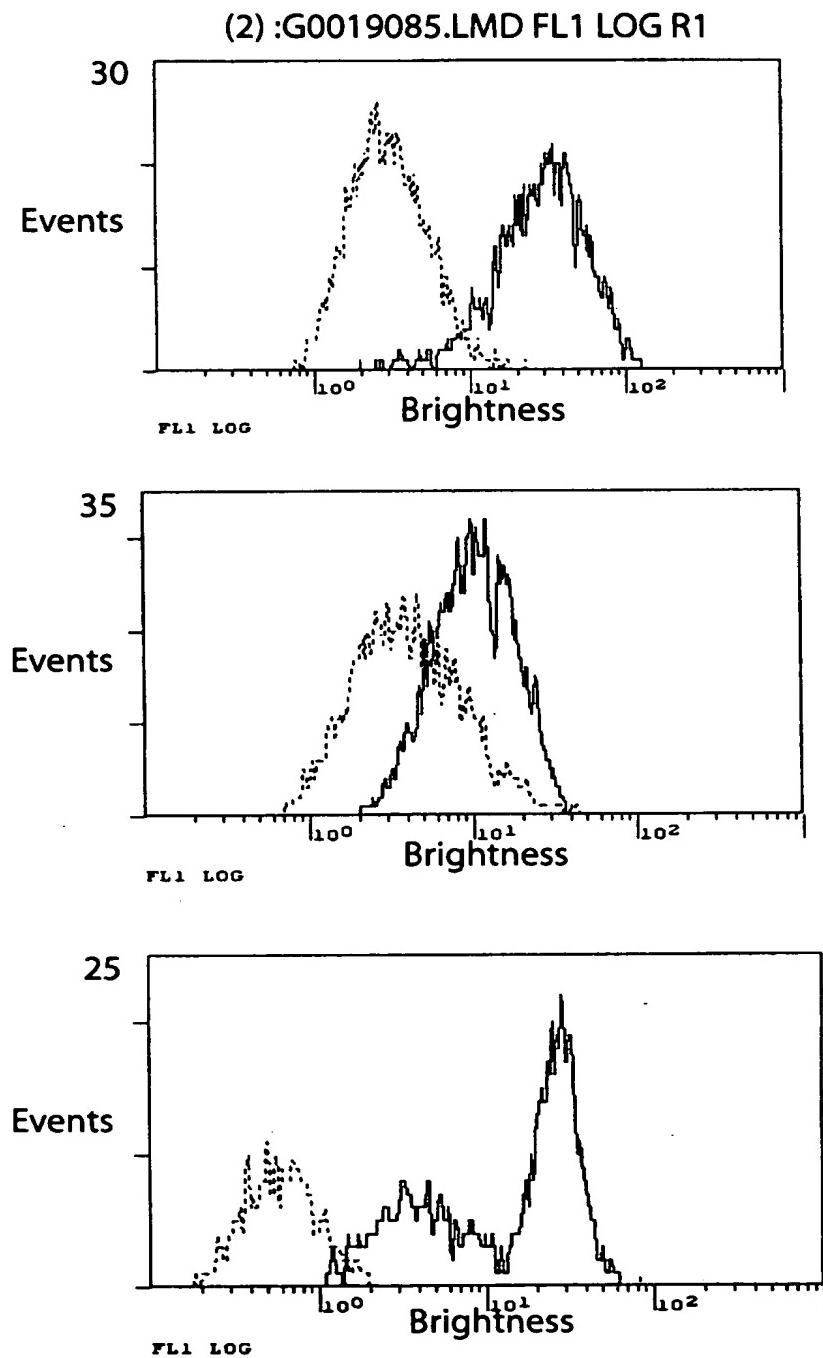
2H7scFv-CD154  
Construct S4  
2H7scFv-CD154  
Construct L2



CD20 CHO cell targets + (control or fusion protein)  
+ Biotin-CD40Ig + PE-SA

**FIG.9**

Induction of Apoptosis Measured by Binding of Annexin V after incubation with 2H7scFv-CD154



.....control supernatant    2H7scFv-CD154 supernatant

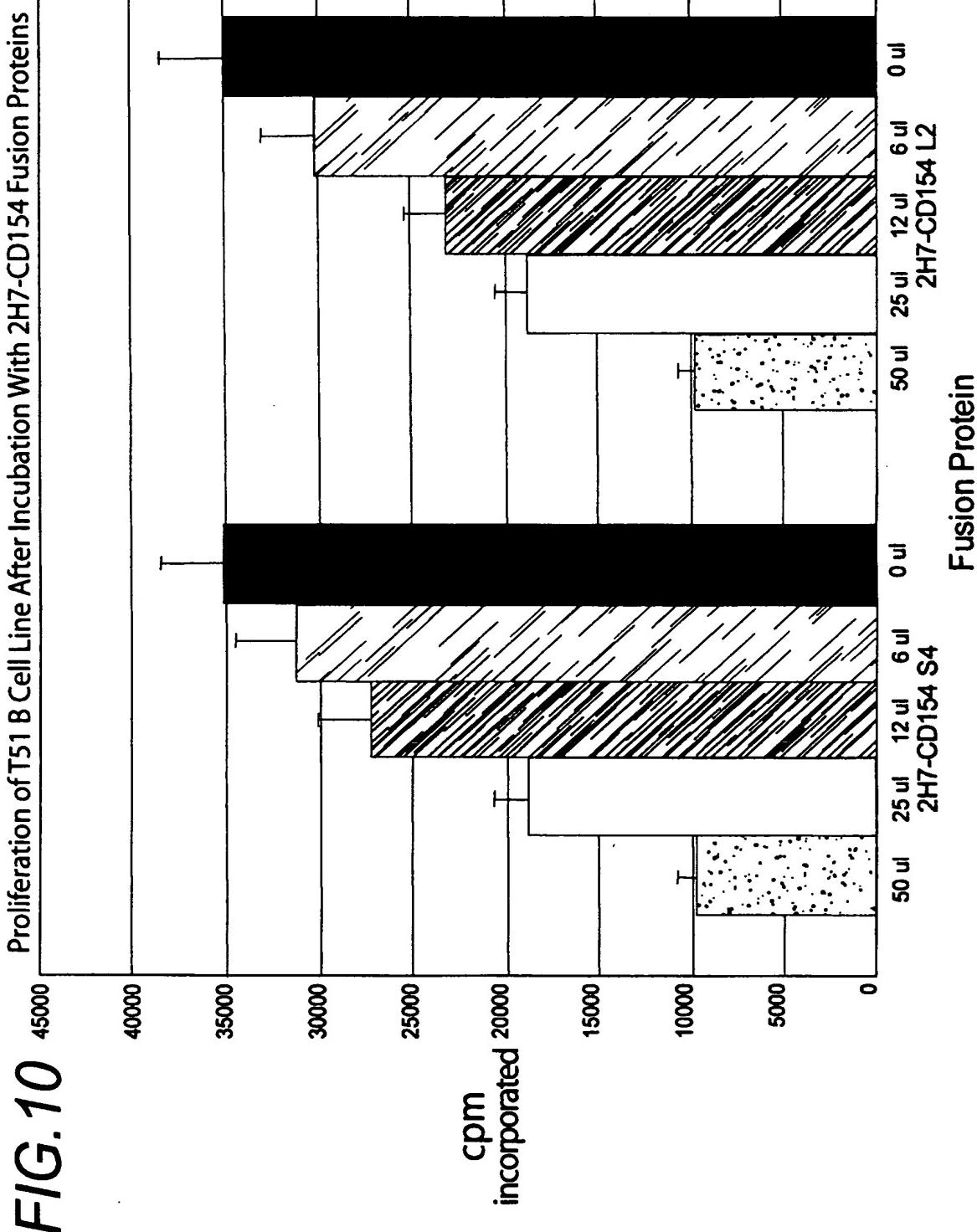
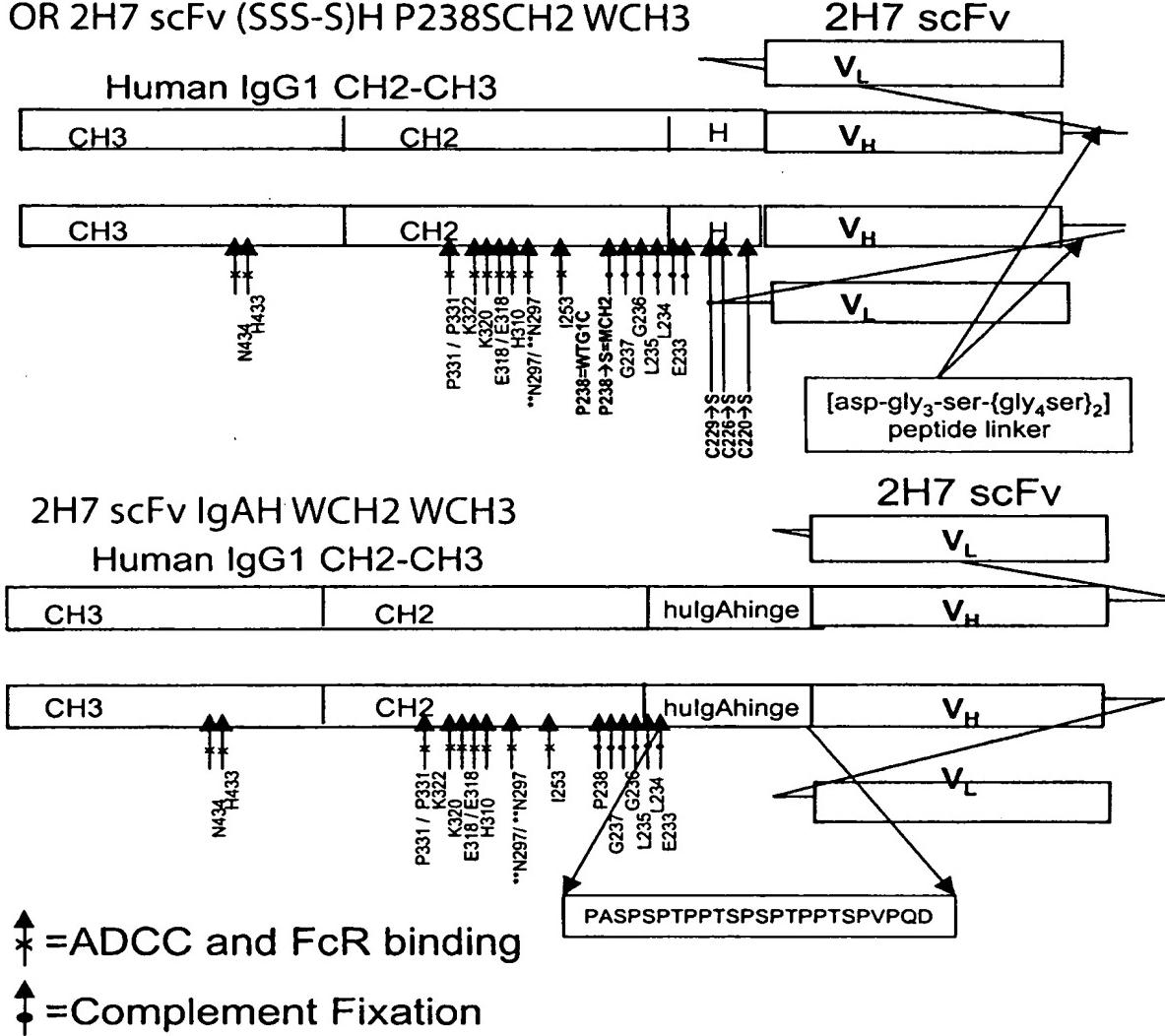
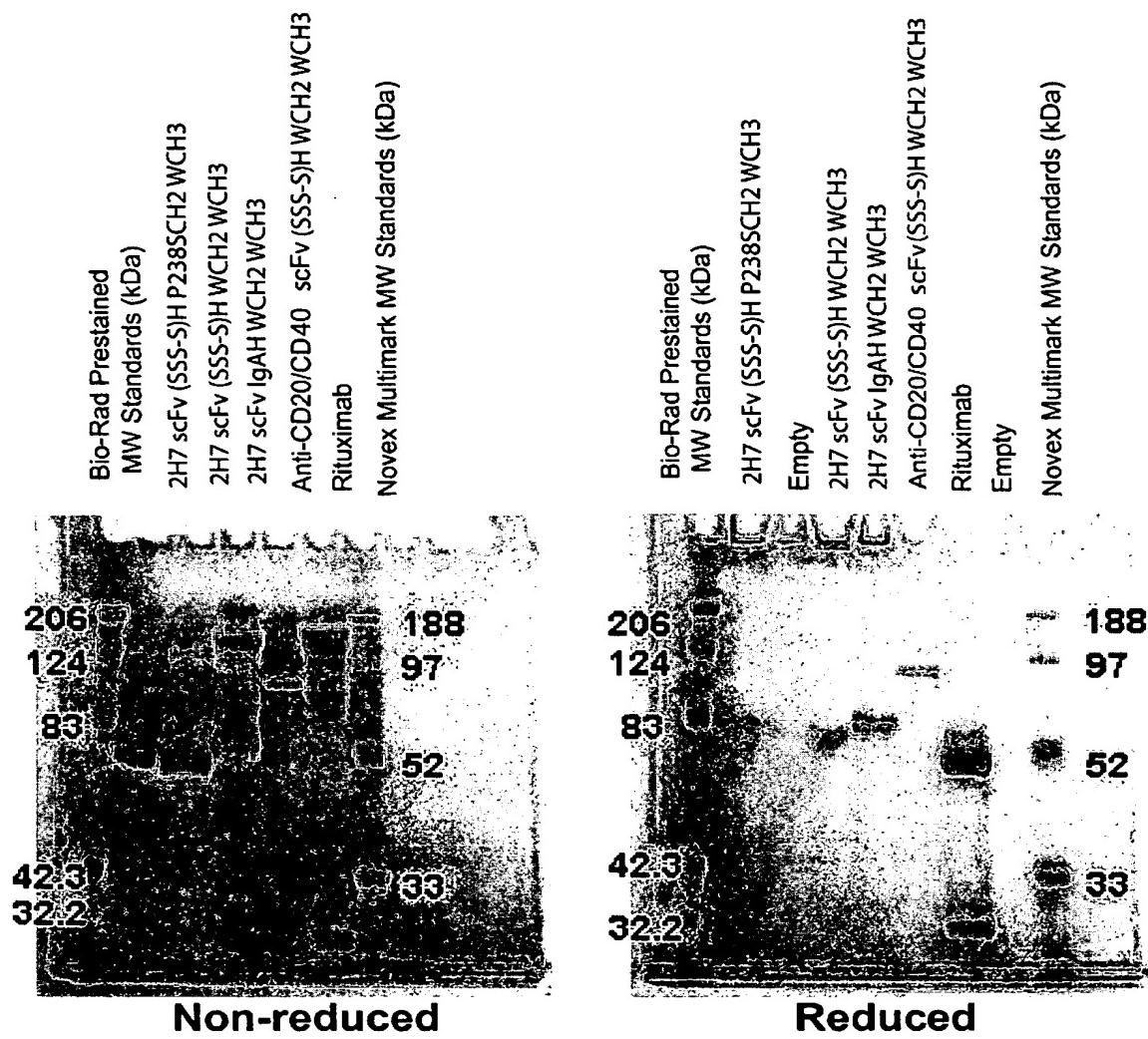
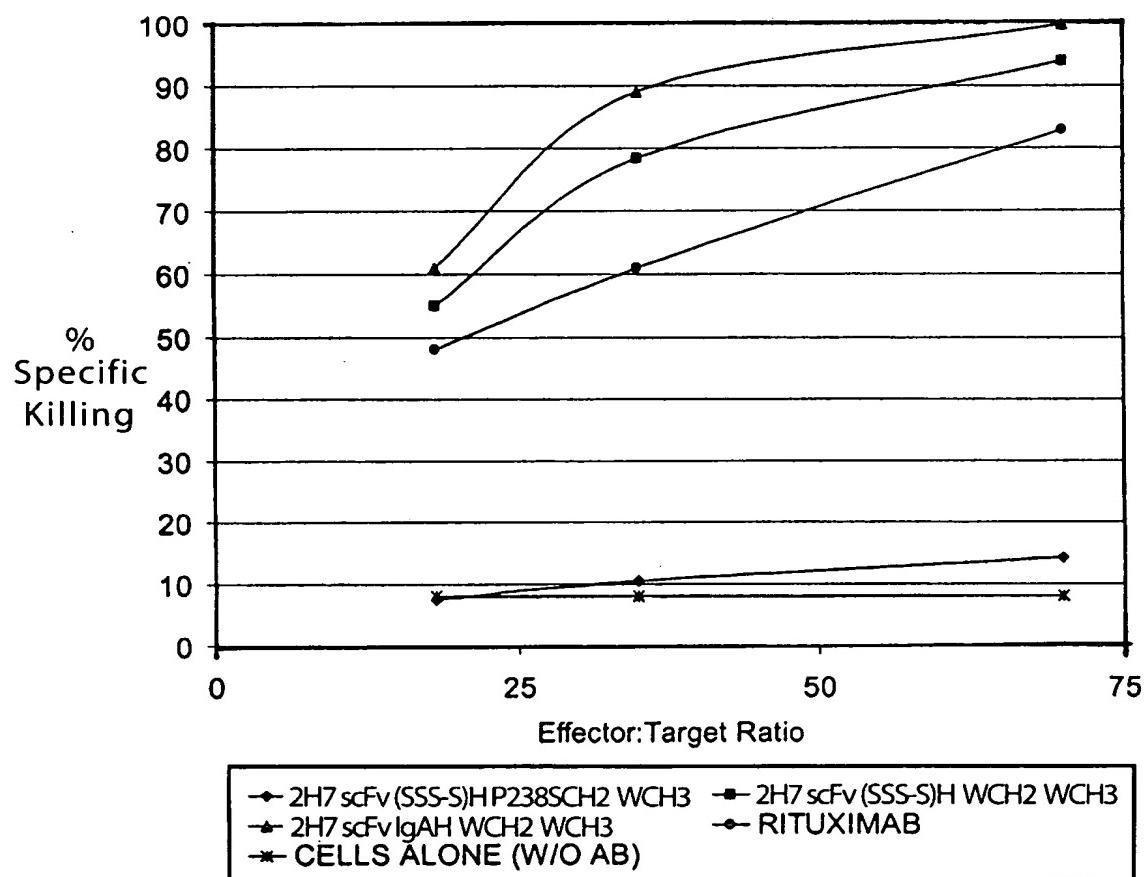


FIG. 11

2H7 scFv (SSS-S)H WCH2 WCH3  
OR 2H7 scFv (SSS-S)H P238SCH2 WCH3

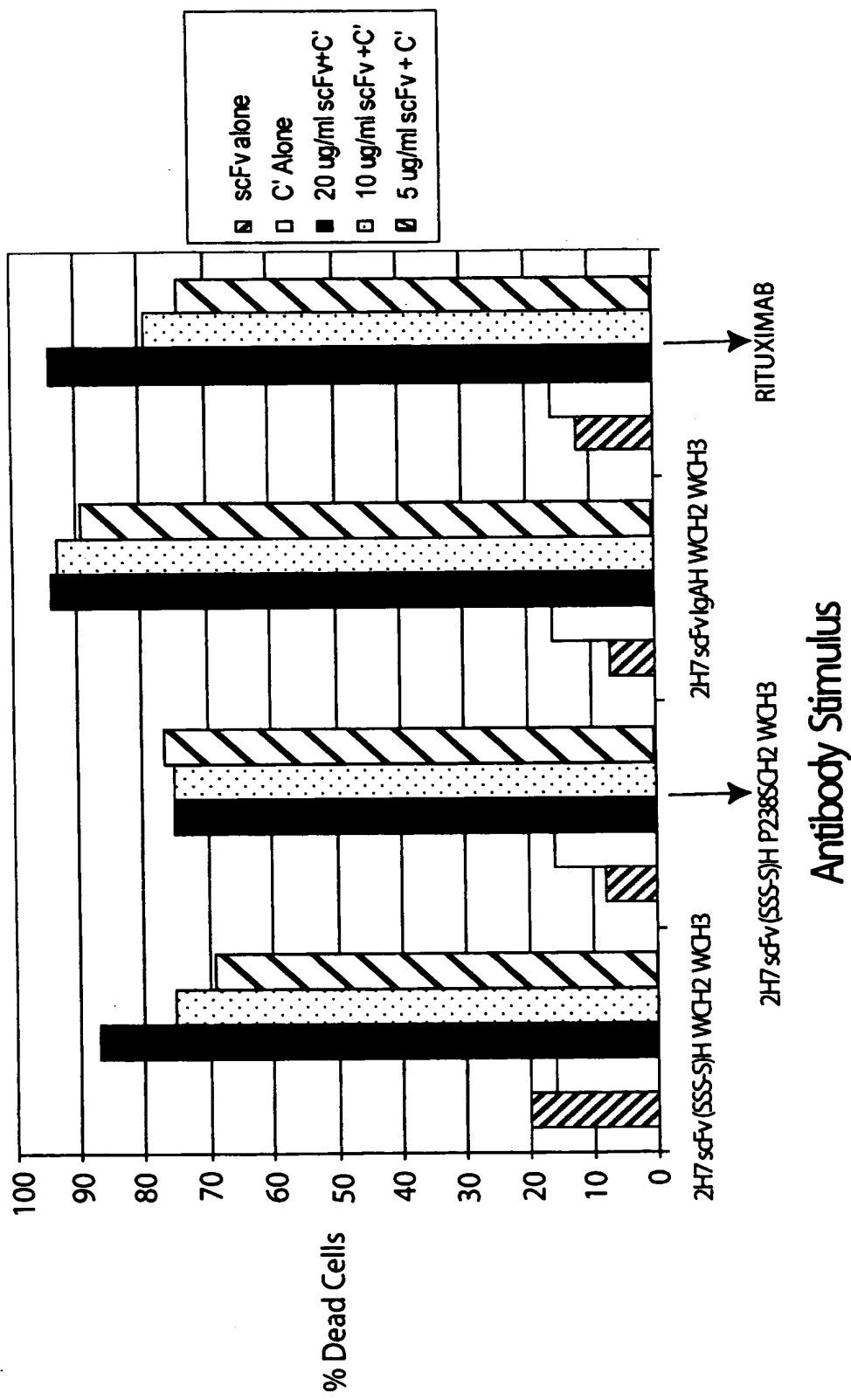


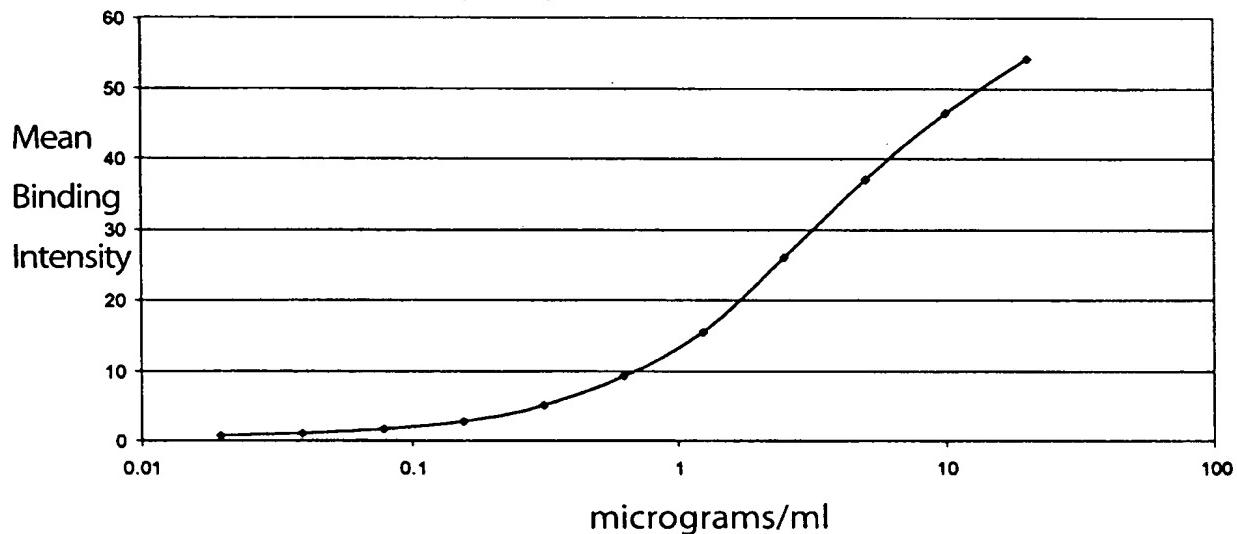
***FIG. 12***

**FIG. 13****ADCC Activity of 2H7 scFv Constructs**

*FIG. 14*

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**FIG. 15****2H7 scFv (SSS-S)H WCH2 WCH3 In Vivo Half Life****2H7 scFv (SSS-S)H WCH2 WCH3 Standard Curve****Macaque A99314**

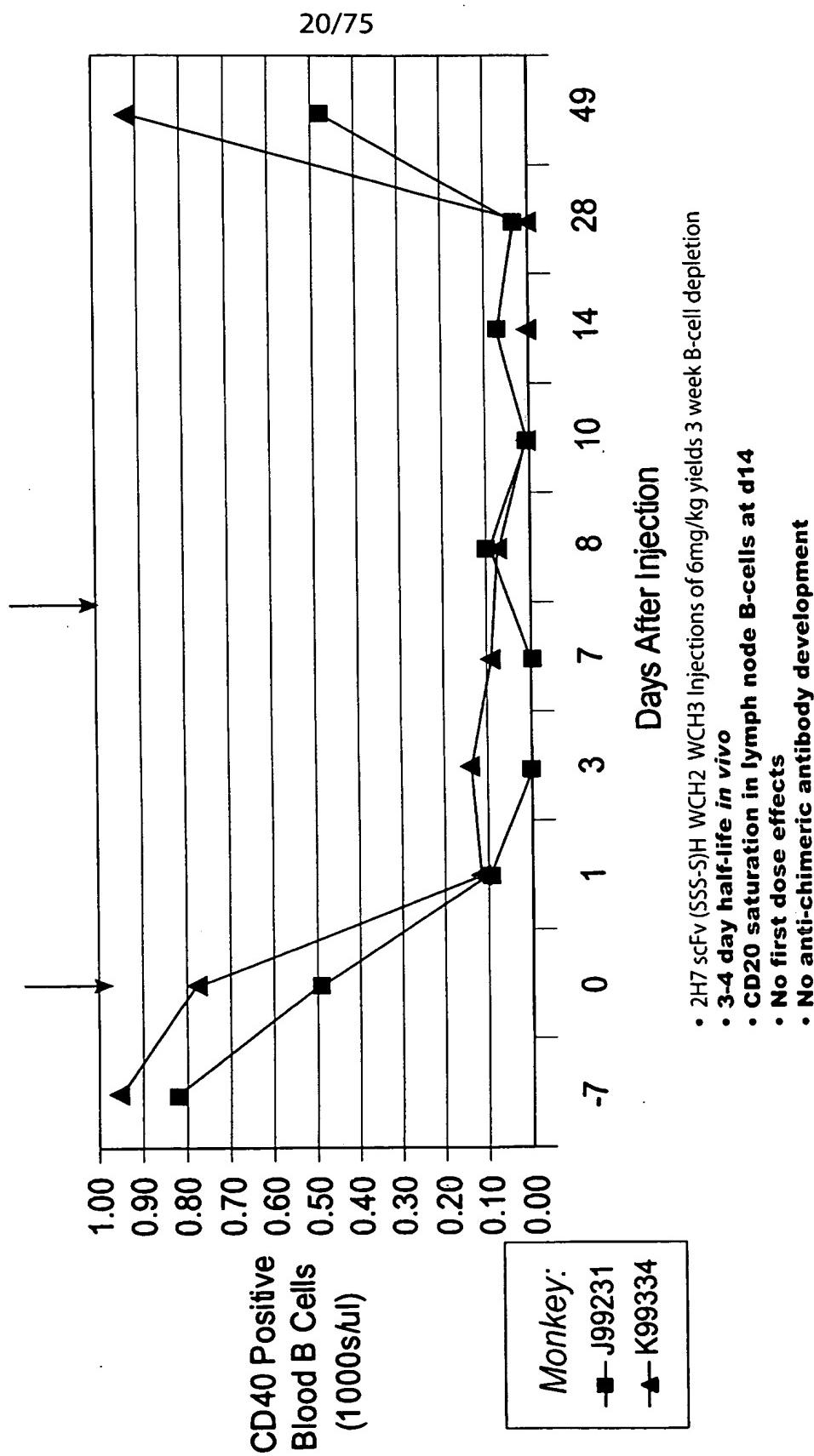
	<u>Day</u>	Binding intensity At 1:50	estimated concentration ( $\mu\text{g}/\text{ml}$ )
Injection #1 →	-7	0.213	<0.1
	0	0.227	<0.1
	1	7.79	25.1
	3	5.51	15.6
	7	3.37	9.4
Injection #2 →	8	11.33	41.7
	10	5.45	15.4
	14	0.27	<0.1

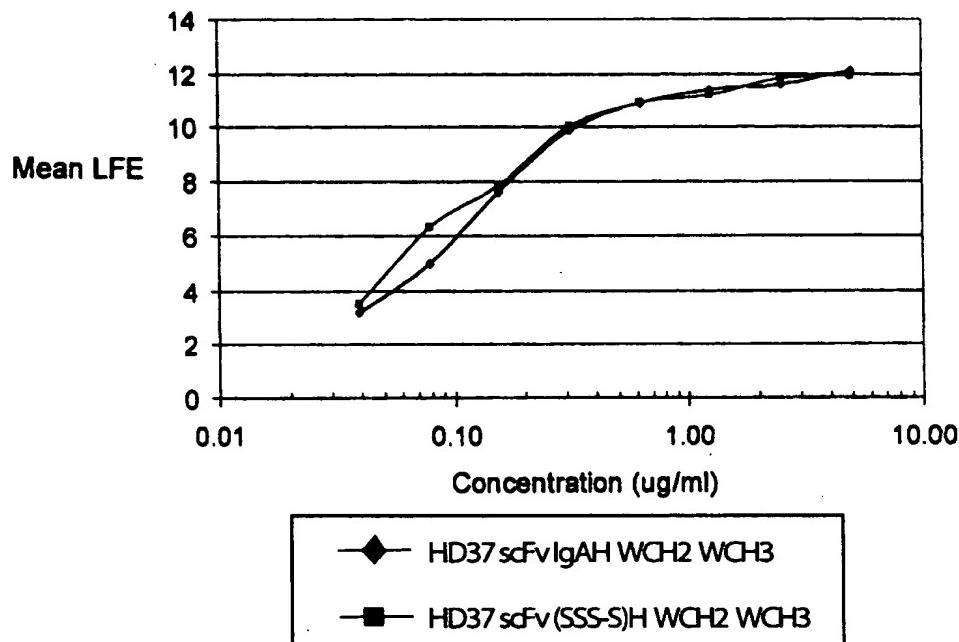
**Macaque F98081**

	<u>Day</u>	Binding intensity At 1:50	estimated concentration ( $\mu\text{g}/\text{ml}$ )
Injection #1 →	-7	0.208	<0.1
	0	0.219	<0.1
	1	6.73	21.9
	3	6.14	19.3
	7	3.04	8.7
Injection #2 →	8	9.83	33.8
	10	4.77	14.4
	14	0.231	<0.1

*FIG. 16*

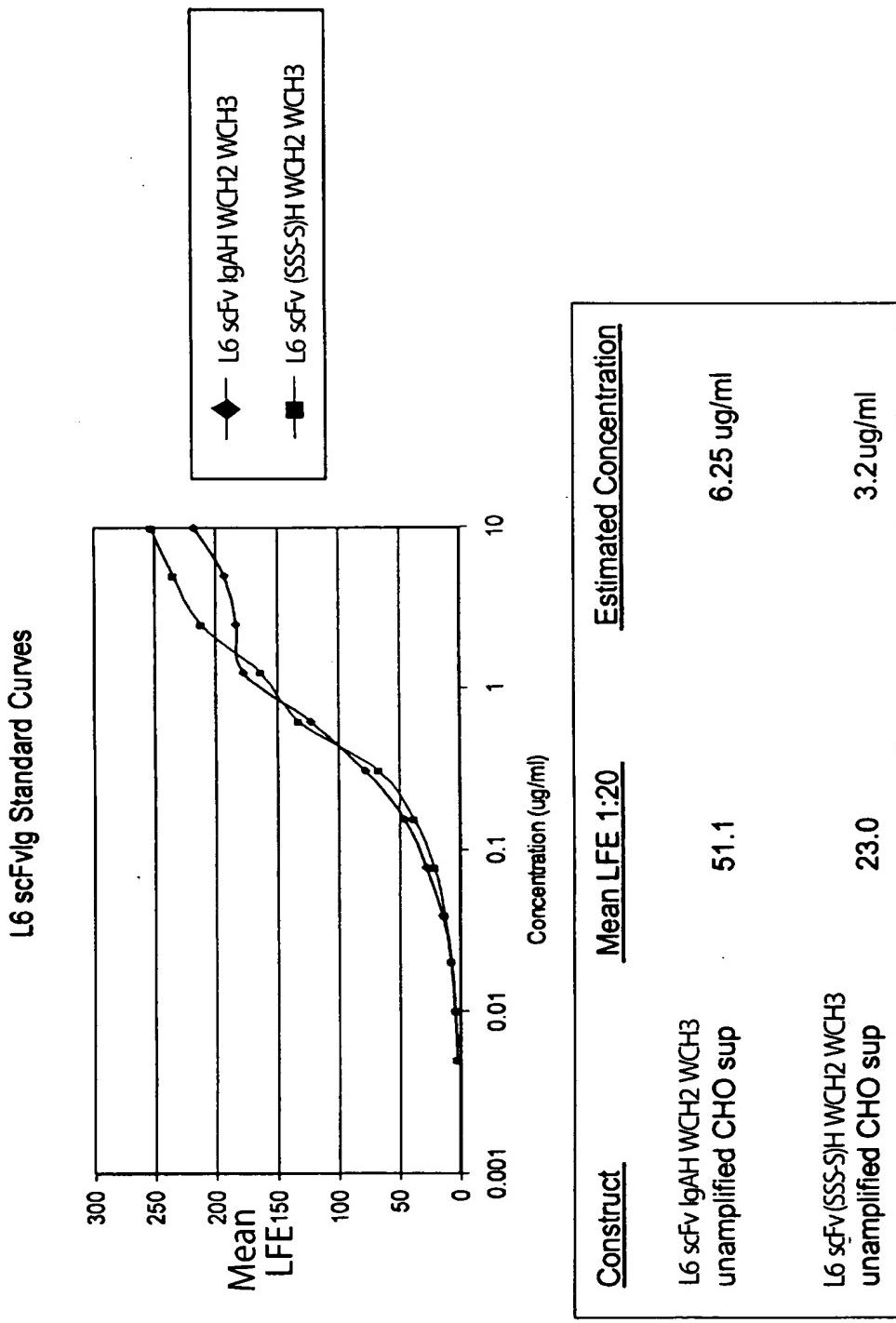
B Cell Depletion in macaques mediated by  
2H7 scFv (SSS-S)H WCH2 WCH3

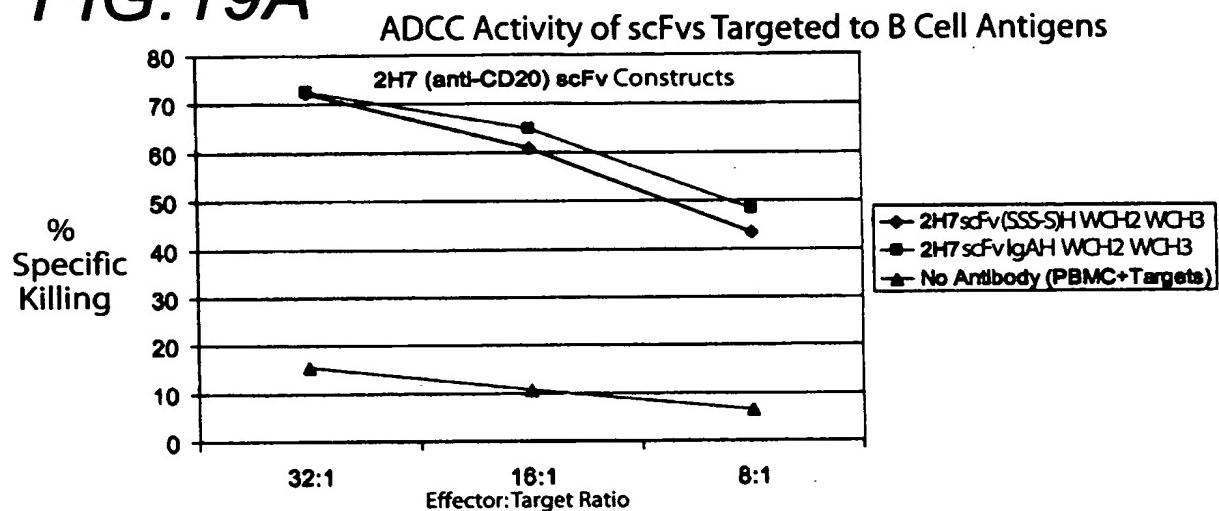
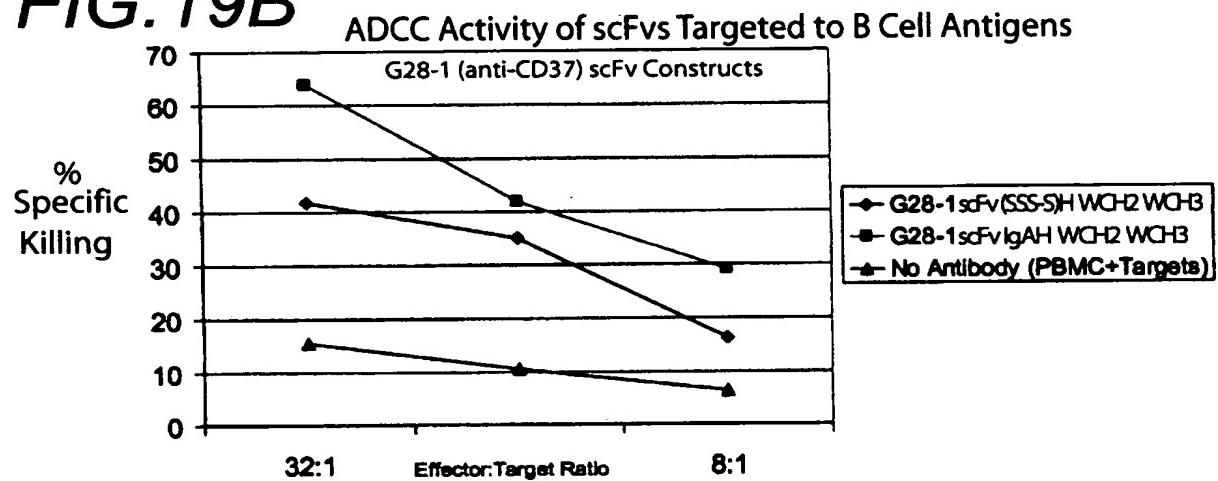
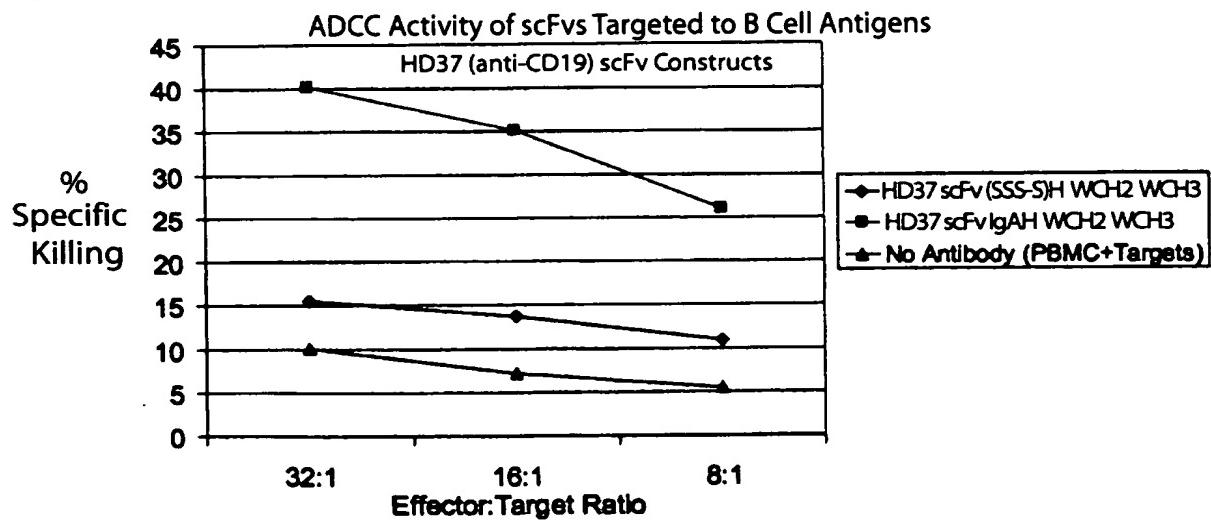


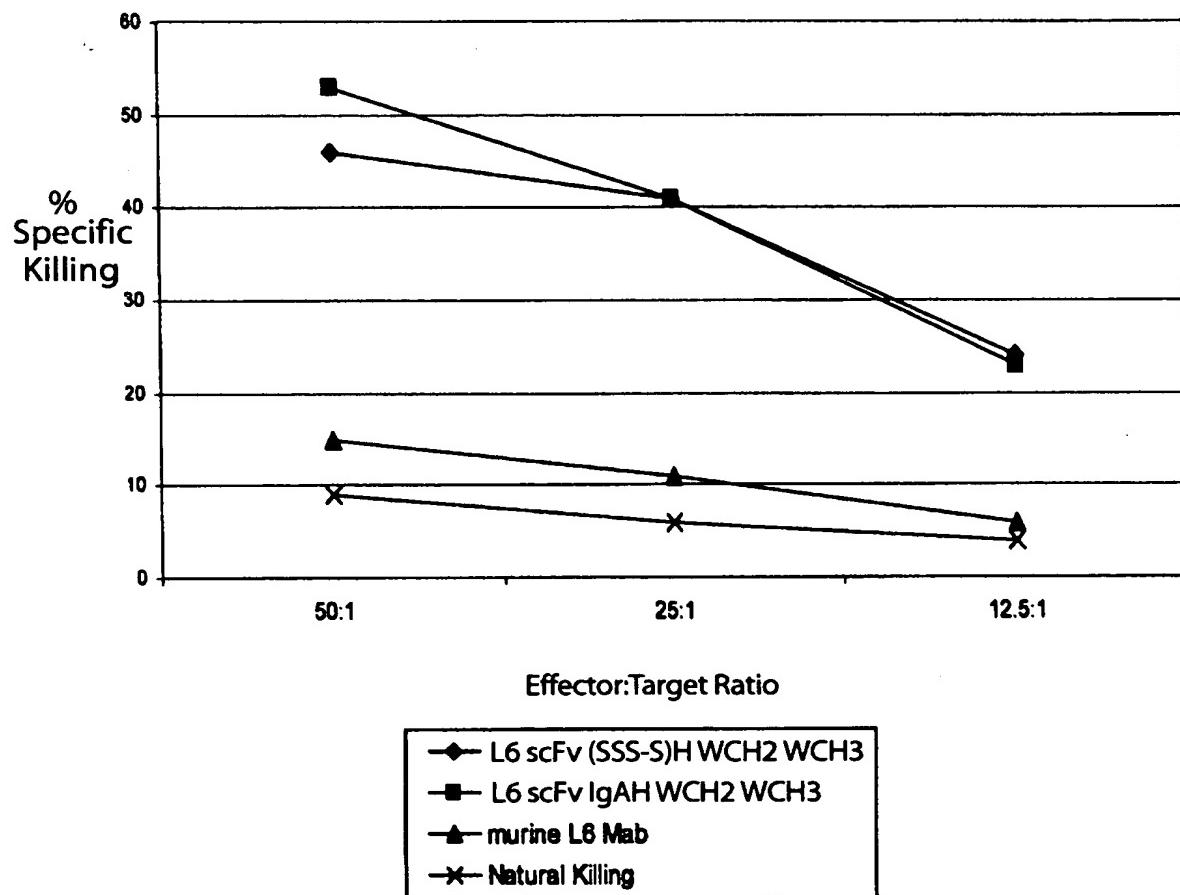
**FIG. 17****Production Levels of HD37 scFv Constructs by CHO Cell Lines****Standard Curve of HD37 scFvIg Constructs  
Binding to B Cells**

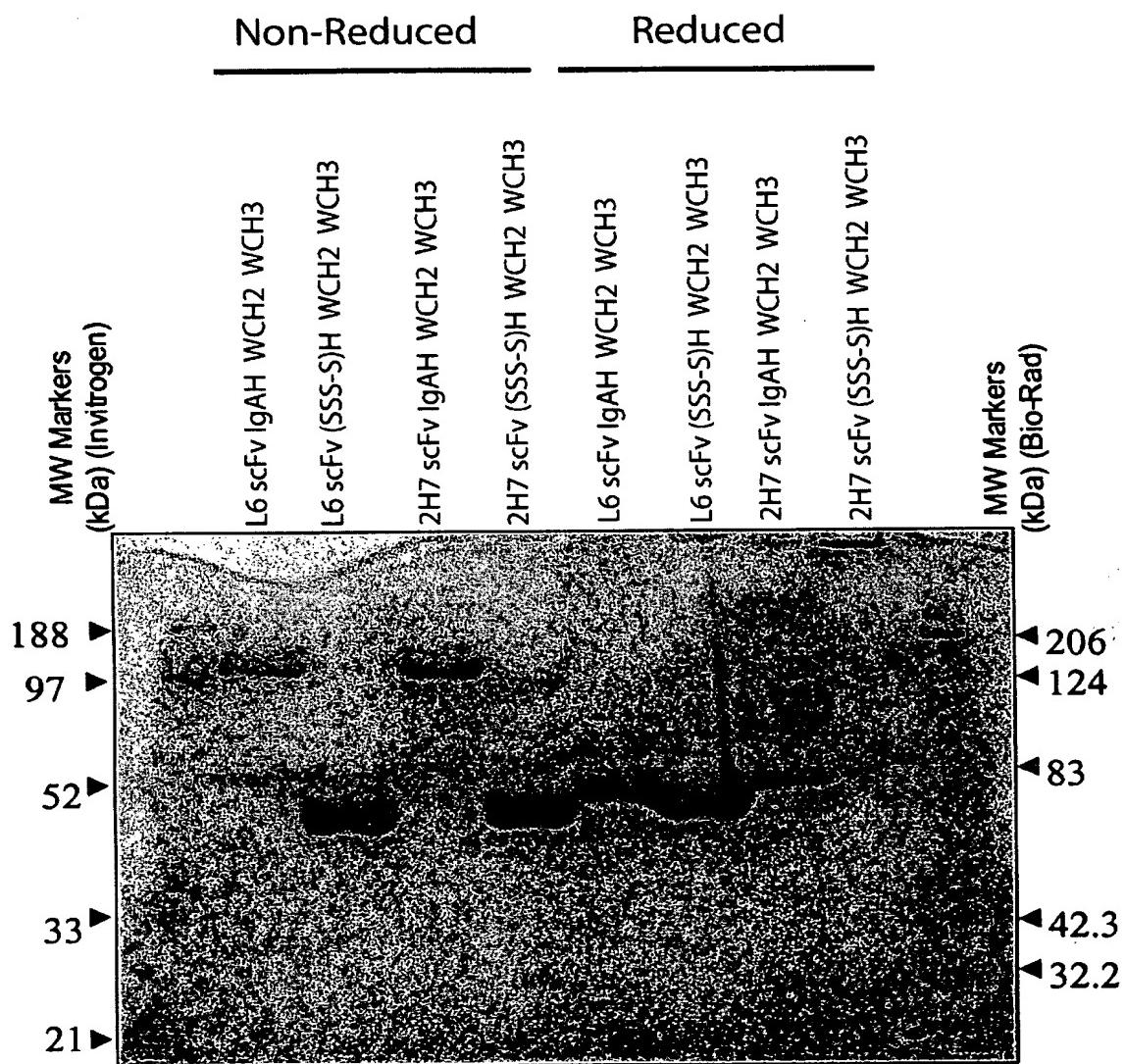
<u>Clone/Isolate</u>	<u>Mean LFE at 1:100</u>	<u>Estimated Concentration</u>
HD37 scFvIgAH WCH2 WCH3	11.2	> 60 ug/ml
1B2	10.4	>50 ug/ml
6C5	10.5	>50 ug/ml
4B1	8.6	>40 ug/ml
HD37 scFv(SSS-S)H WCH2 WCH3	10.9	> 50 ug/ml
2G8	10.6	> 50 ug/ml
3F3	8.3	>40 ug/ml
3D9	11.1	> 60 ug/ml

**FIG. 18** Production of L6 scFvlg Constructs by CHO Cells



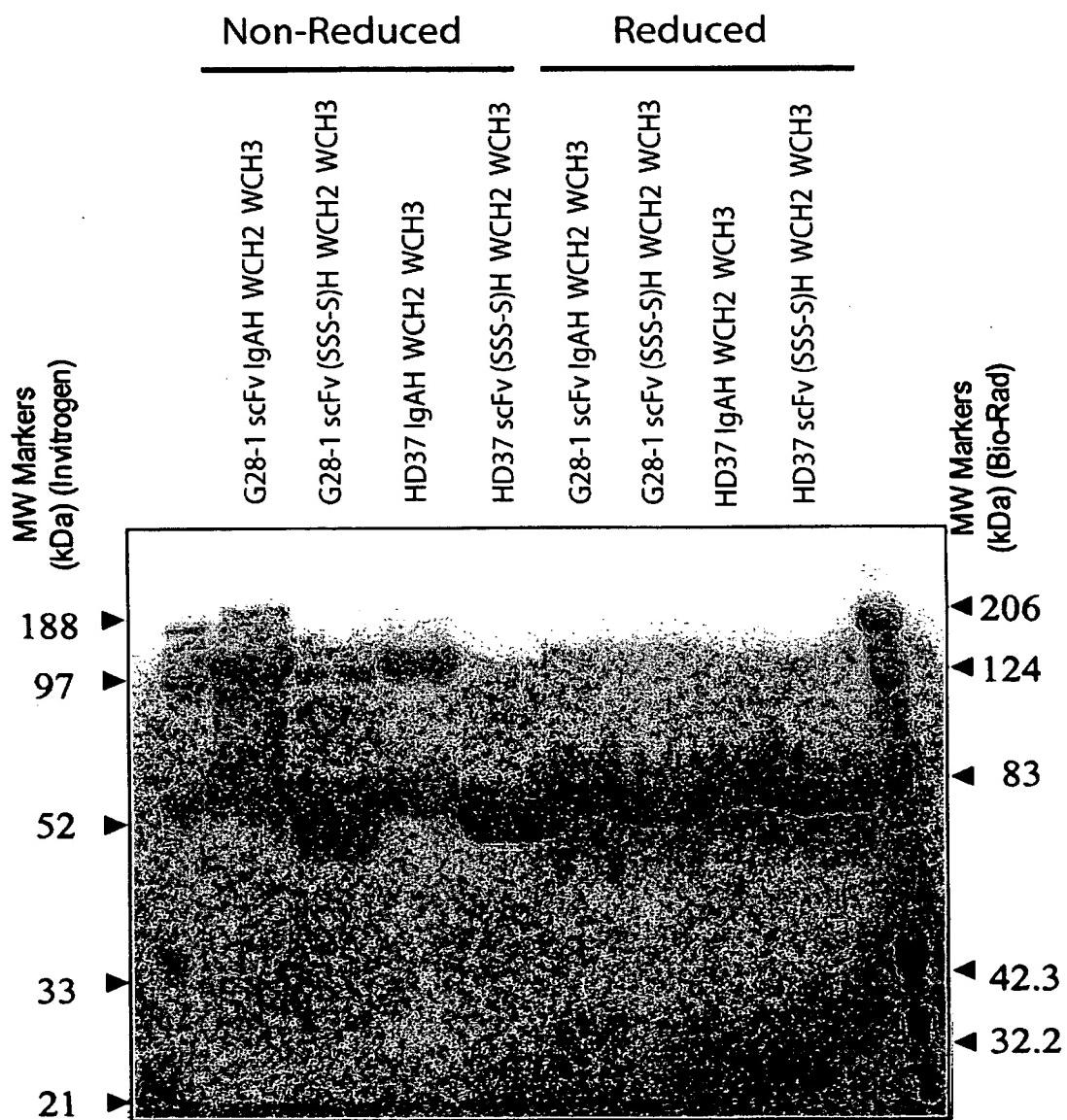
**FIG. 19A****FIG. 19B****FIG. 19C**

**FIG.20****ADCC Activity of L6 scFvIg Constructs****ADCC Activity of L6scFvIg Constructs with 2981 Targets**

**FIG. 21****SDS-PAGE Analysis of L6 and 2H7 scFvIg Constructs**

**FIG.22**

**SDS-PAGE Analysis of G28-1 and HD37  
scFvIg Constructs**



# FIG. 23

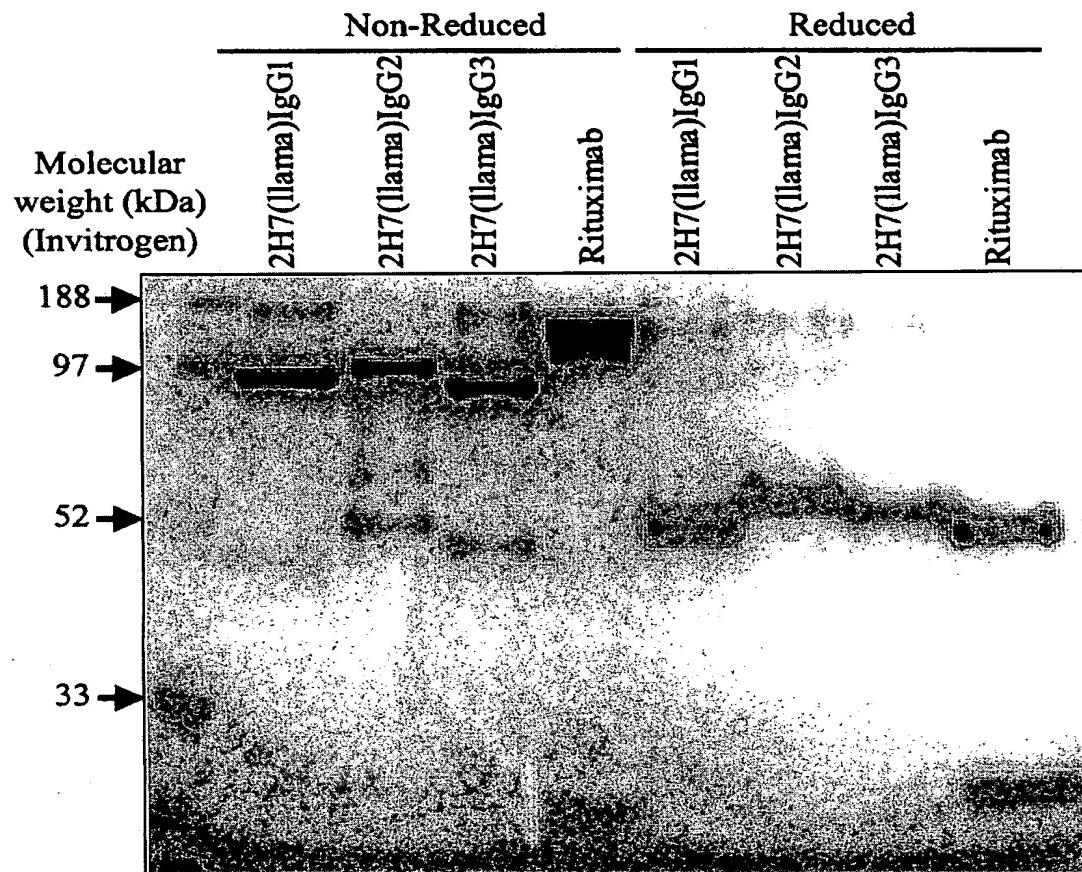
Sequence alignment of human and Llama Fc regins.

HINGE                    CH2 →

Human IgG1:	DQEPKSCDKT-----	HTCPPC	PAPELLGGPSVFLFPPKPKDTLMSIRTPEVTCAVVDVSHEDPEVKFNWYVDG
Llama IgG2:	DQEPKTPKPQPOQPOQPNPTTESKCPKC	PAPELLGGPSVFIFPPKPKDVLISIGRPEVTCAVVDVGQEDPEVSFNWYIDG	
Llama IgG1:	--EPHGG-----	CTCPQC	PAPELLGGPSVFIFPPKPKDVLISIGRPEVTCAVVDVGKEDPEVNFMNWFIDG
Llama IgG3:	--AHHSEDP-----	SKCPKO	PGPELLGGPPTVFIFFPKAKDVLISITRKPEVTCLWWTWVKKTLRSSSSWSVDD

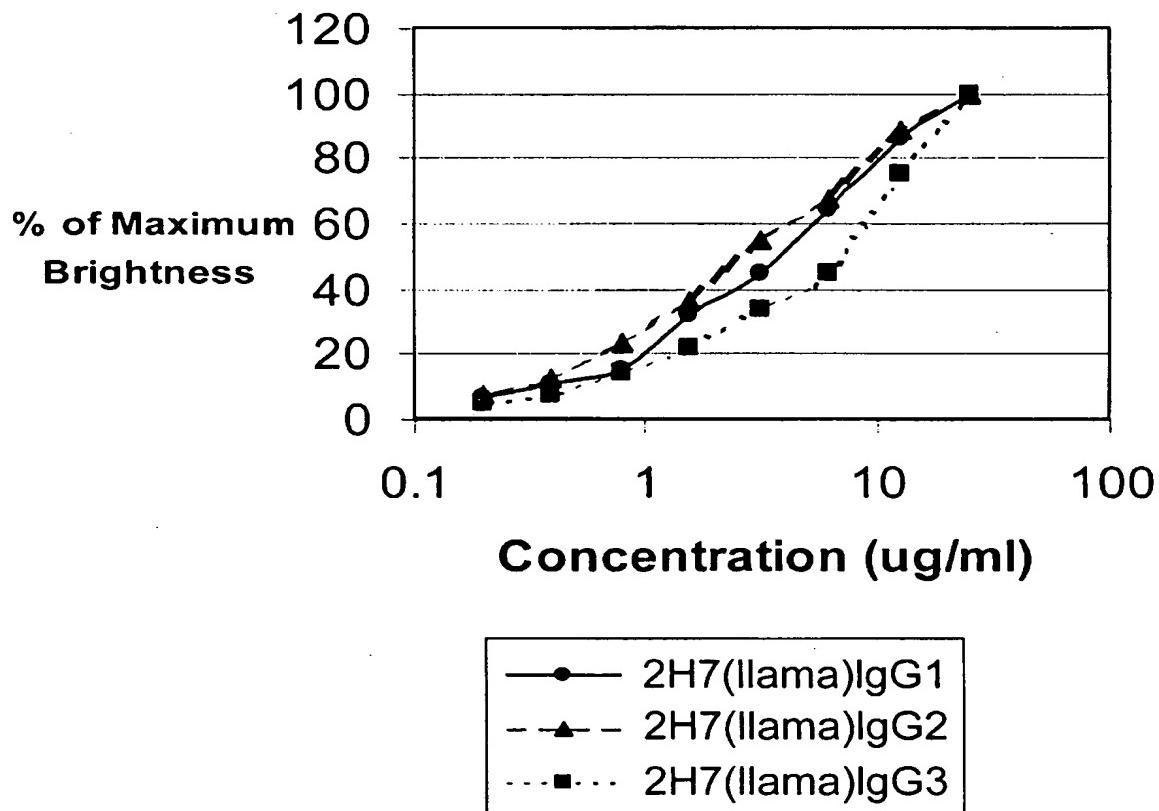
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAGQPREPQVYTLPPSRDELTKNQVSILT  
TAEVRANTRPKKEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNIKALPAPIEKTIISKAGQTREPQVYTLAPFHREELAKDTVSVT  
VEVRTANTKPKKEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNIKALPAPIERTISKAGQTREPQVYTLAPFHREELAKDTVSVT  
TEVHTAETKPKKEQFNSTYRVVSVLPIQHQDWLTGKEFKCKVNNIKALPAPIERTISKAGQTREPQVYTLAPFHREELAKDTVSVT

CLVKGFYPSDIAVEWESNGOPEN--NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSYMHEALHNHYTOKSLSLSPGK  
CLVKGFYPPDINNEWQRNGQPESXGTYATTTPQLDNDGTYFLXSKXSVGKNTWQQGETFTCVVMHEALHNHYTOKSITQSSGK  
CLVKGFYPADINNEWQRNGQPESEGTYANTPPQLDNDGTYFLYSRLSVDGKNTWQRGETLTGVVMHEALHNHYTOKSITQSSGK  
CLVKGF F PADINNEWQRNGQPESEGTYANTPPQLDNDGTYFLYSKLSVGKNTWQQGEVFTCVVMHEALHNHSTQKSITQSSGK

**FIG.24**

**FIG. 25**

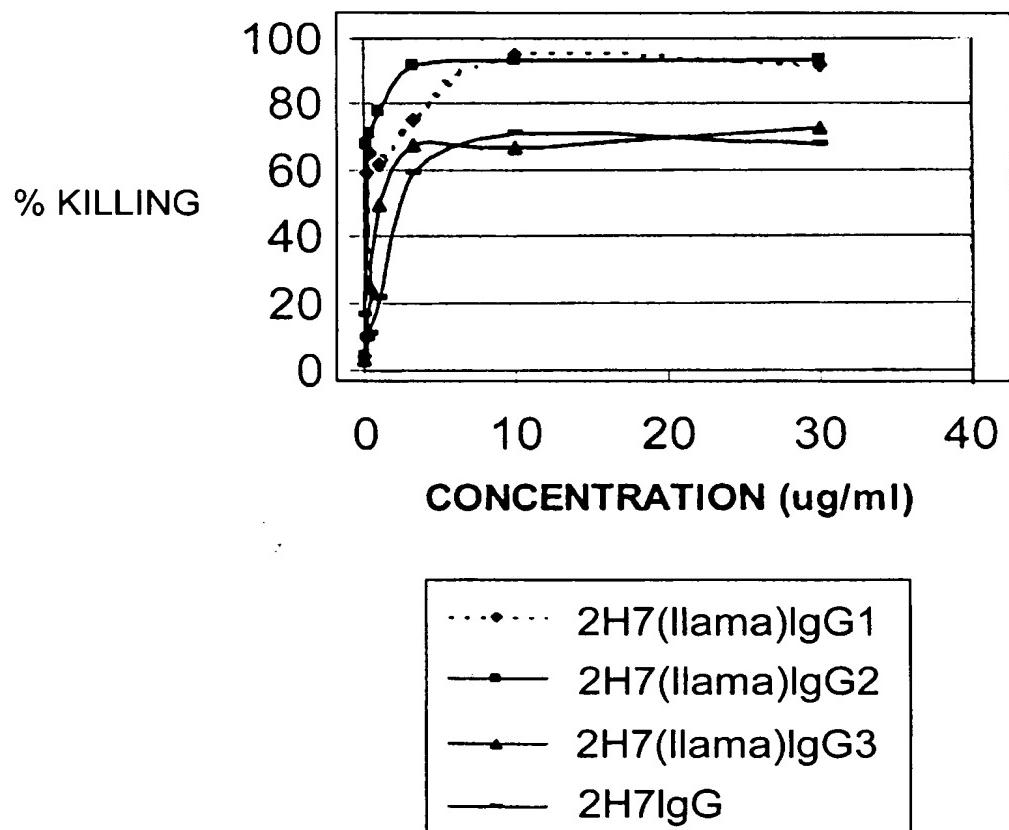
## Llama Tails Binding Assay with CD20 CHO Cells

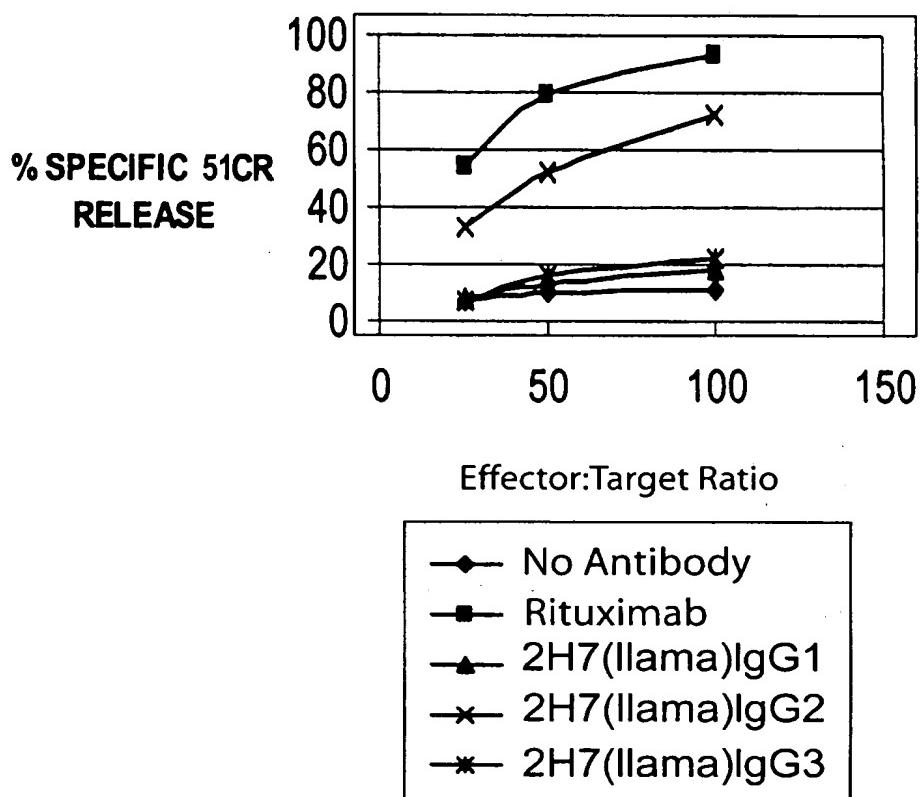


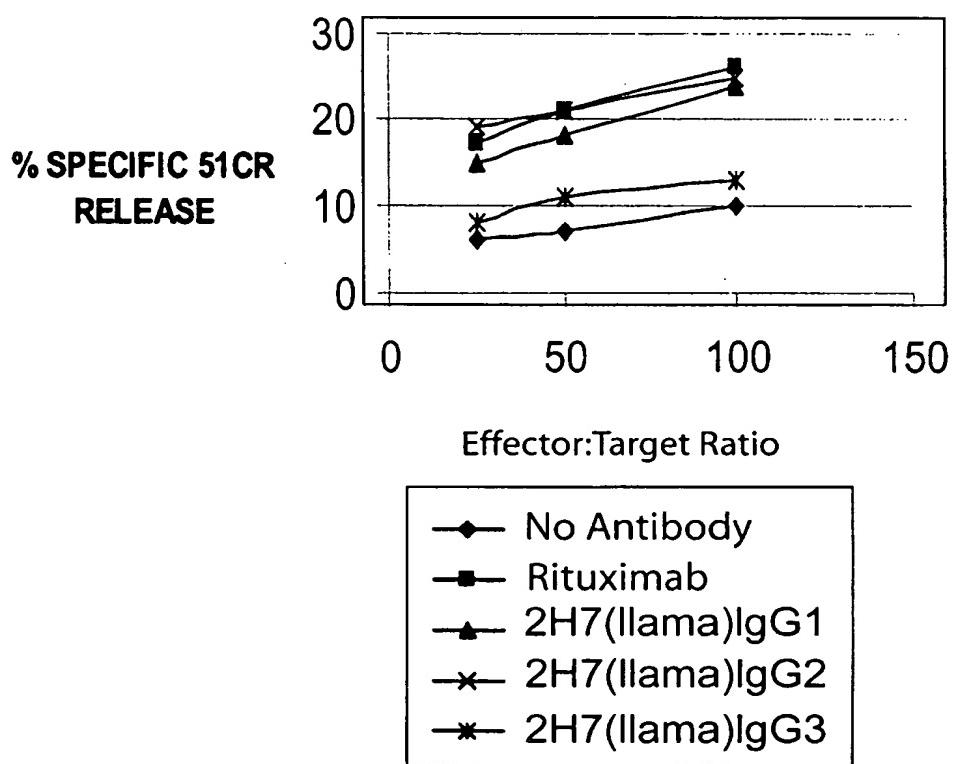
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## FIG.26

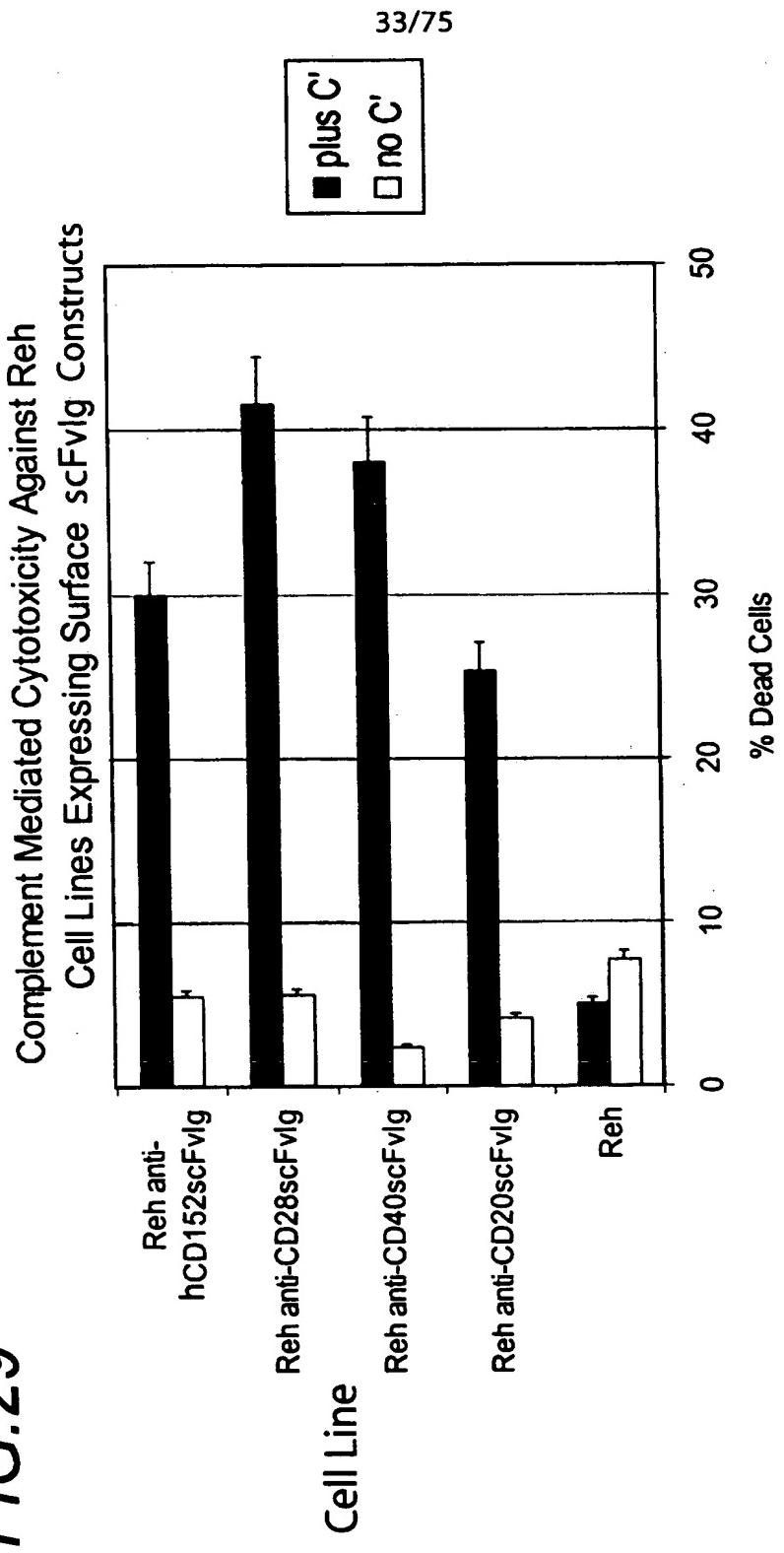
### 2H7 scFv Llama Constructs Complement Assay with BJAB Cells



**FIG.27****ADCC ASSAY WITH BJAB TARGETS  
AND HUMAN PBMC EFFECTORS**

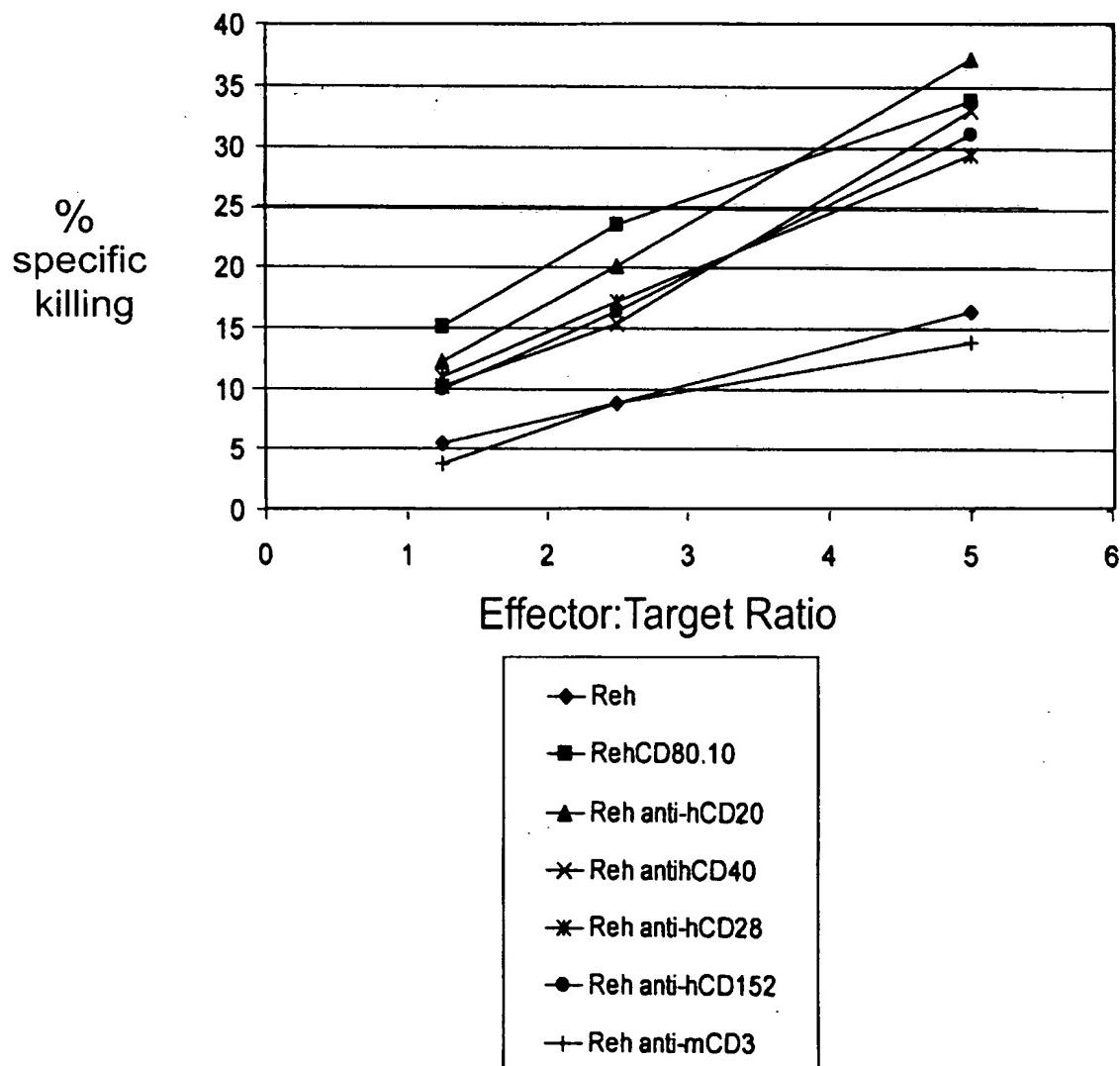
**FIG.28****ADCC ASSAY WITH BJAB TARGETS  
AND LLAMA PBMC EFFECTORS**

*FIG. 29*



**FIG. 30**

## ADCC Activity of Cell Surface Expressed ScFvIg Constructs



# FIG. 31

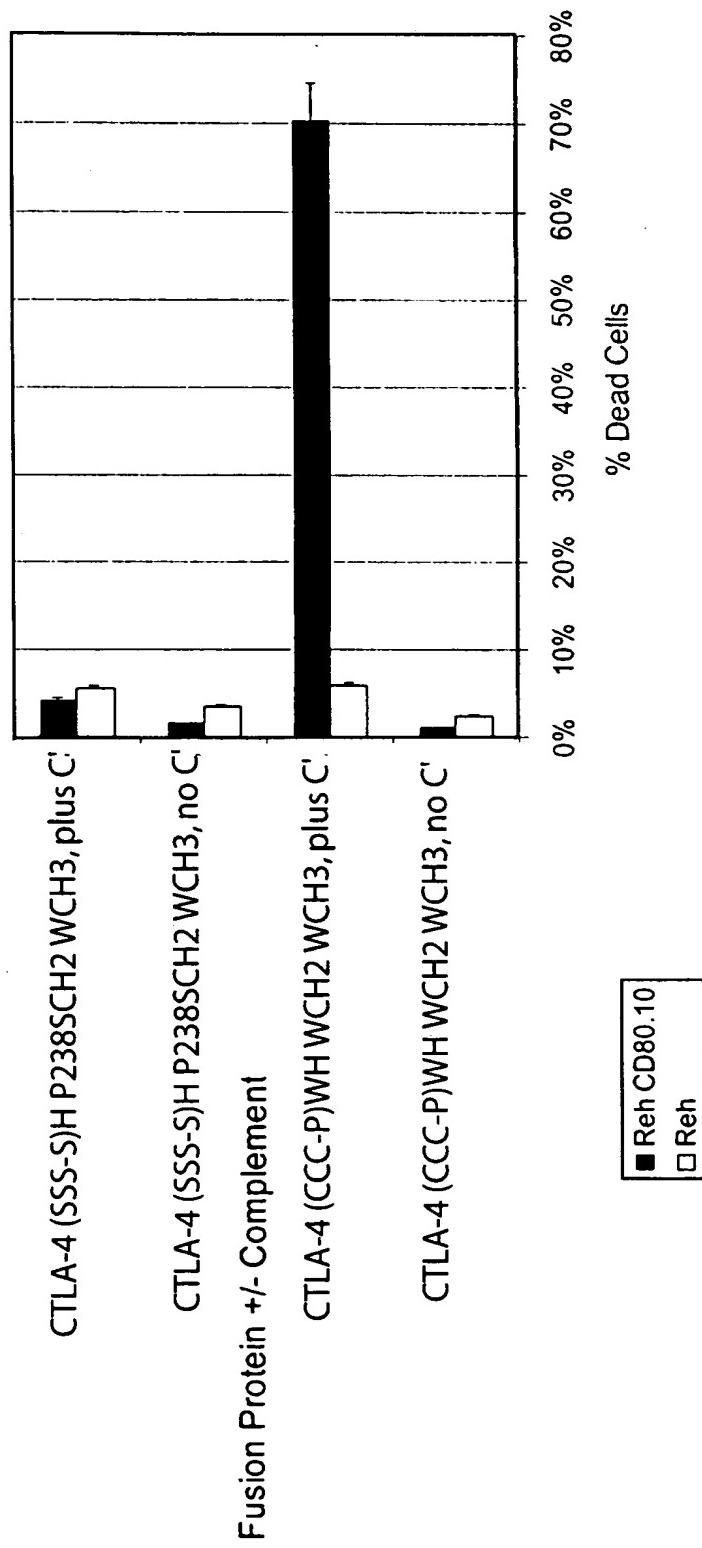
## Ig Constructs and Nomenclature:

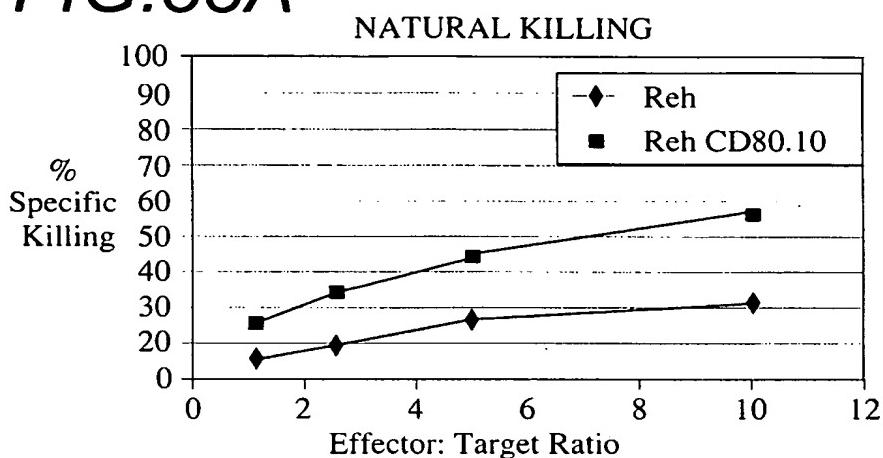
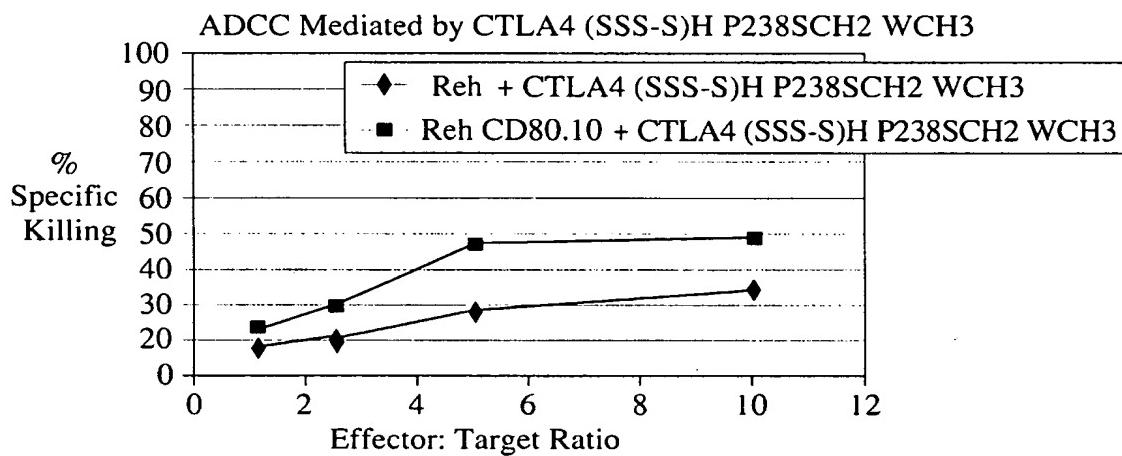
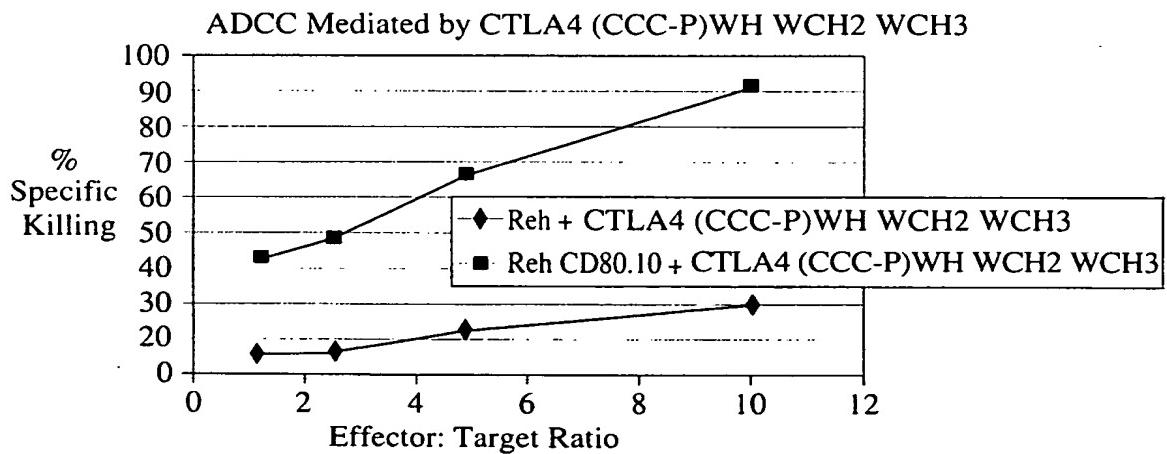
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Name Identifier	Hinge Sequence	CH2 Sequence	CH3 Sequence
(CCC-P)WH WCH2 WCH3	IgG1 WT Hinge (CCC)	Wild Type CH2	Wild Type CH3
(SSS-SH) WCH2 WCH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
VHL11S (SSS-SH) WCH2 WCH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
(SSC-P)H WCH2 WCH3	IgG1 Mutant Hinge (SSC)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
(SCS-SH) WCH2 WCH3	IgG1 Mutant Hinge (SCS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
(CSS-SH) WCH2 WCH3	IgG1 Mutant Hinge (CSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
(SSSS-SH) P238SCH2WCH3	IgG1 Mutant Hinge (SSS)	Mutant CH2 (IgG1) Pro→Ser 238	Wild type CH3 (IgG1)
IgAH WCH2 WCH3	IgA Hinge	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)
IgAH IgA CH2CH3	IgA Hinge	Wild type CH2 (IgA)	Wild type CH3 (IgA)
IgAH IgA CH2T4CH3	IgA Hinge	Wild type CH2 (IgA)	Truncated CH3 (IgA) Missing 4 aa at COOH

# FIG. 32

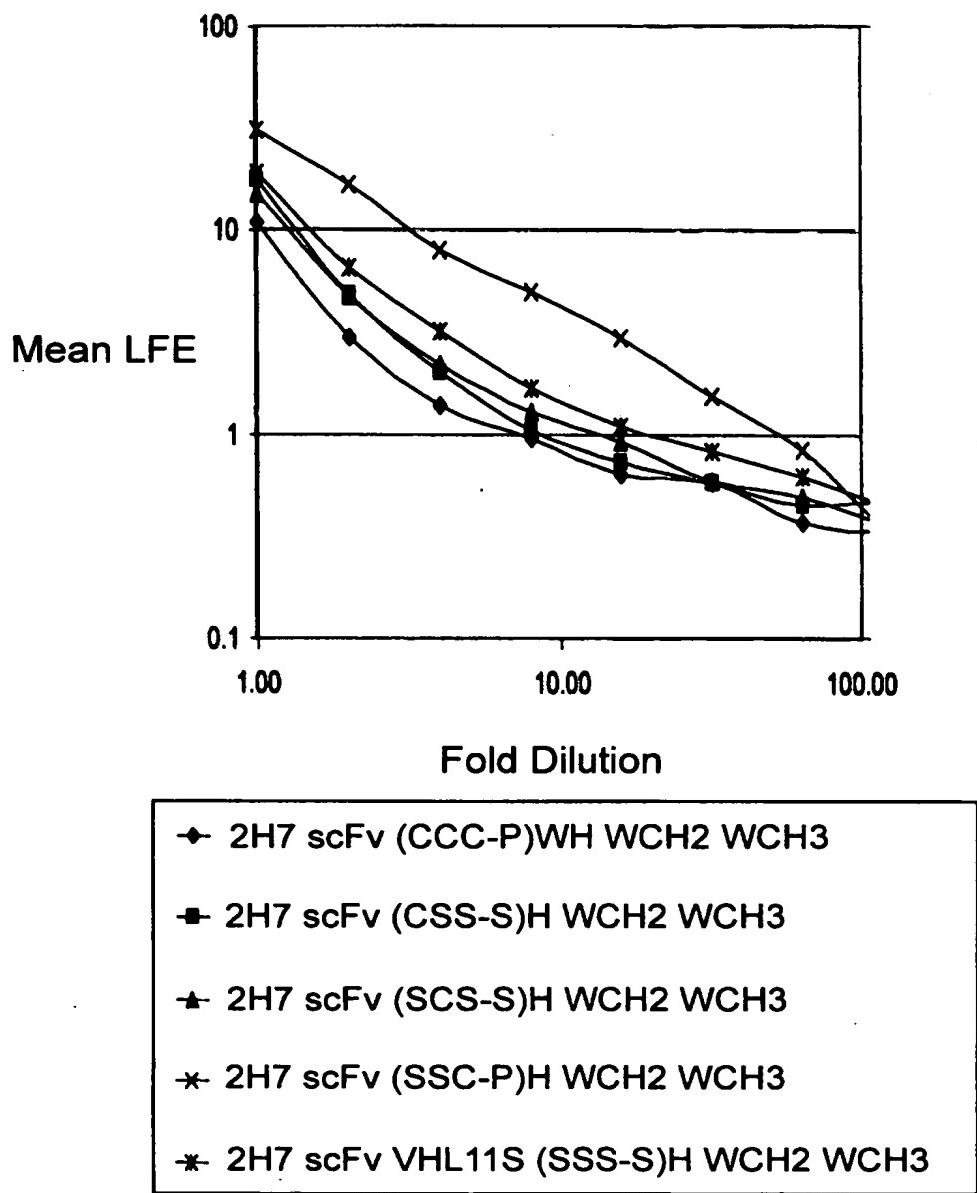
CDC Activity of CTLA4Ig Constructs against Reh and Reh CD80 Transfected Cells

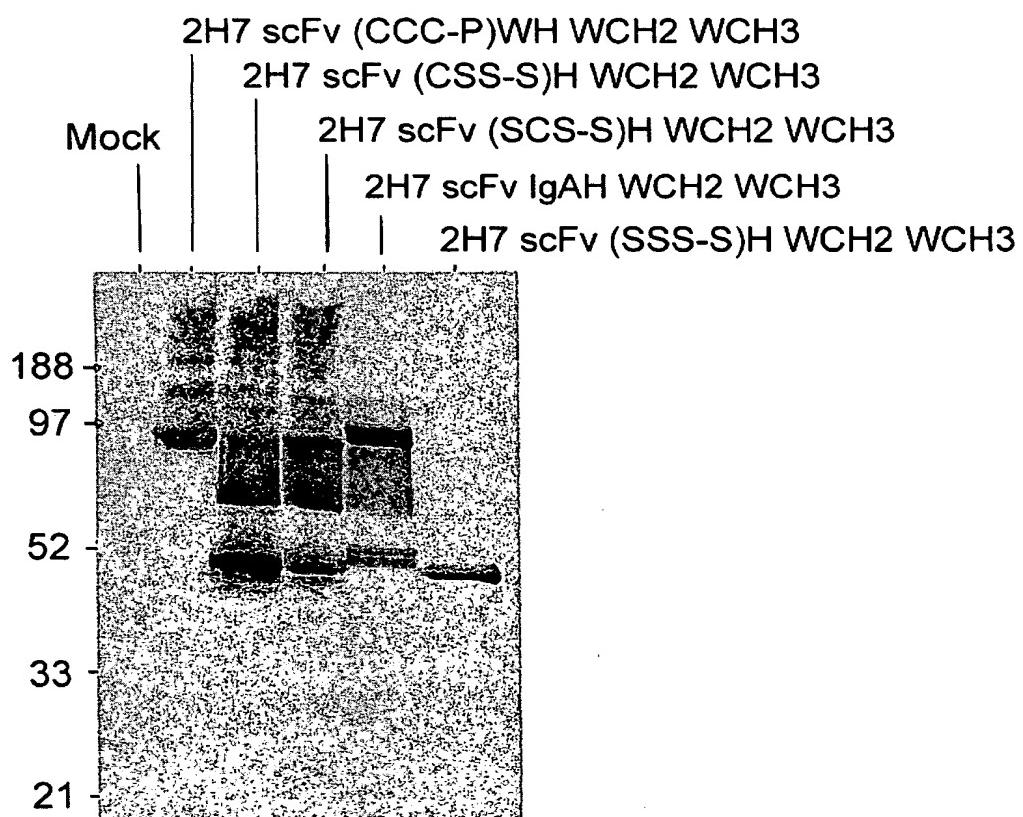


**FIG.33A****FIG.33B****FIG.33C**

**FIG.34**

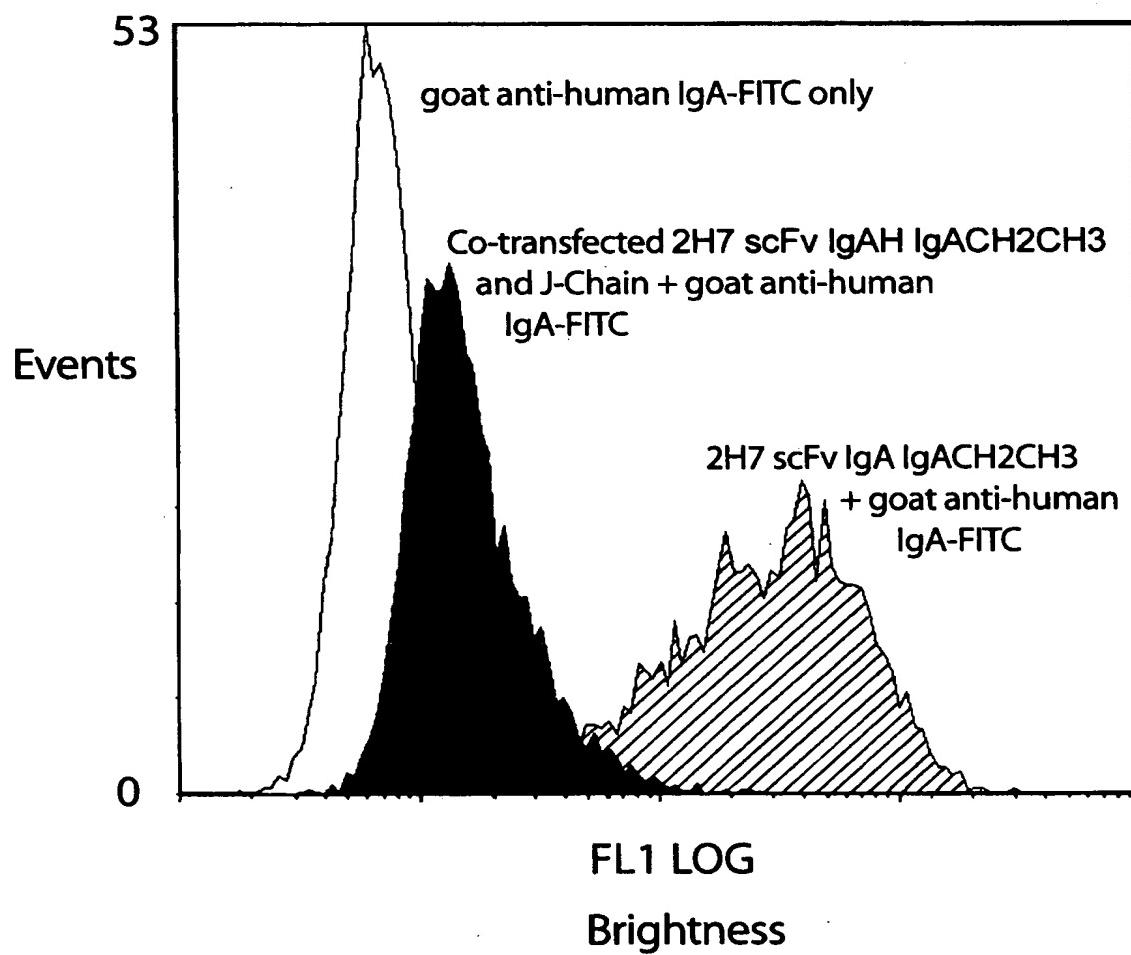
## Binding of Constructs Alternative to CD2 CHO Cells

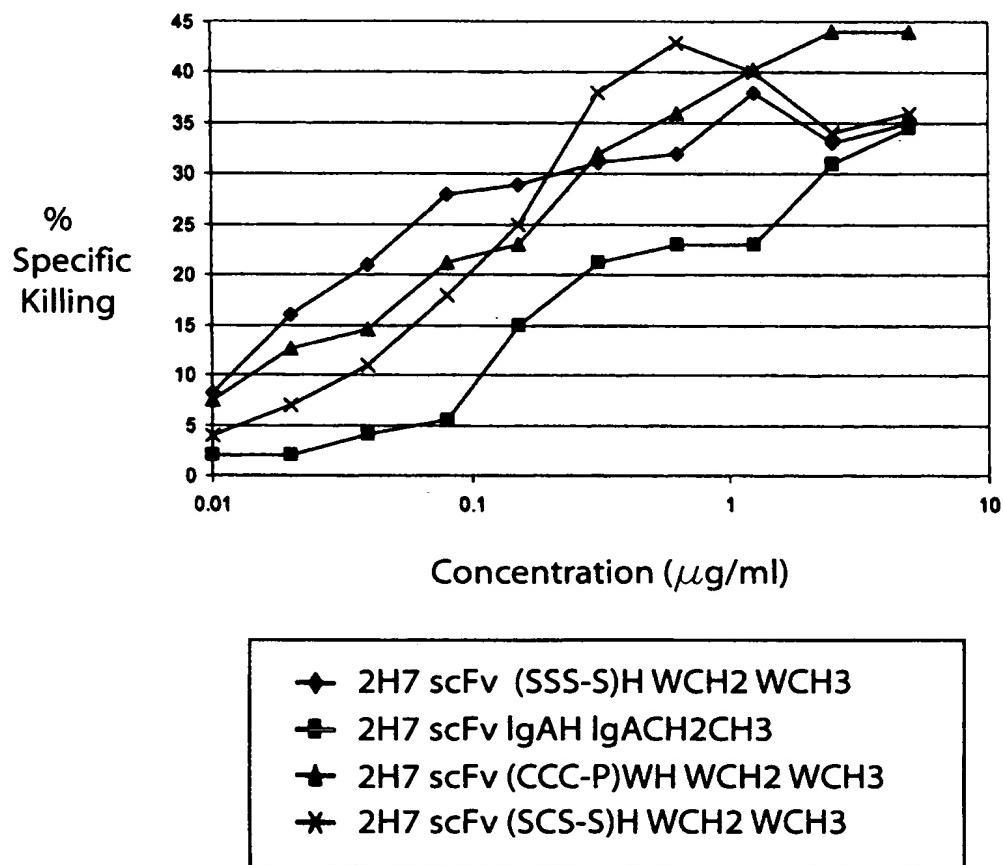


**FIG.35**

**FIG. 36**

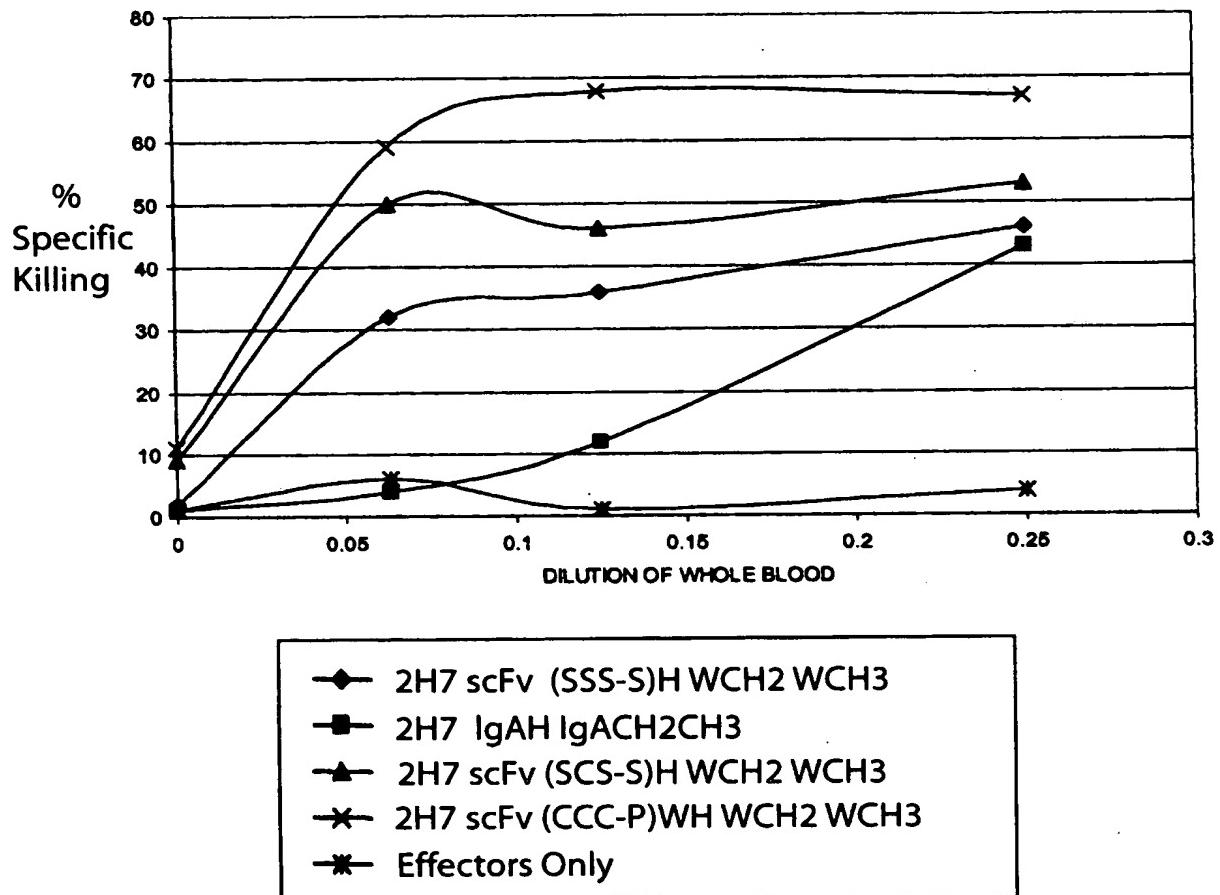
Binding to CD20 CHO cells by Constructs  
that link anti-CD20 scFv to IgA Fc Domains



**FIG.37****Titration of CD20 Specific scFvIg Constructs for ADCC Activity Using Whole Blood Effectors**

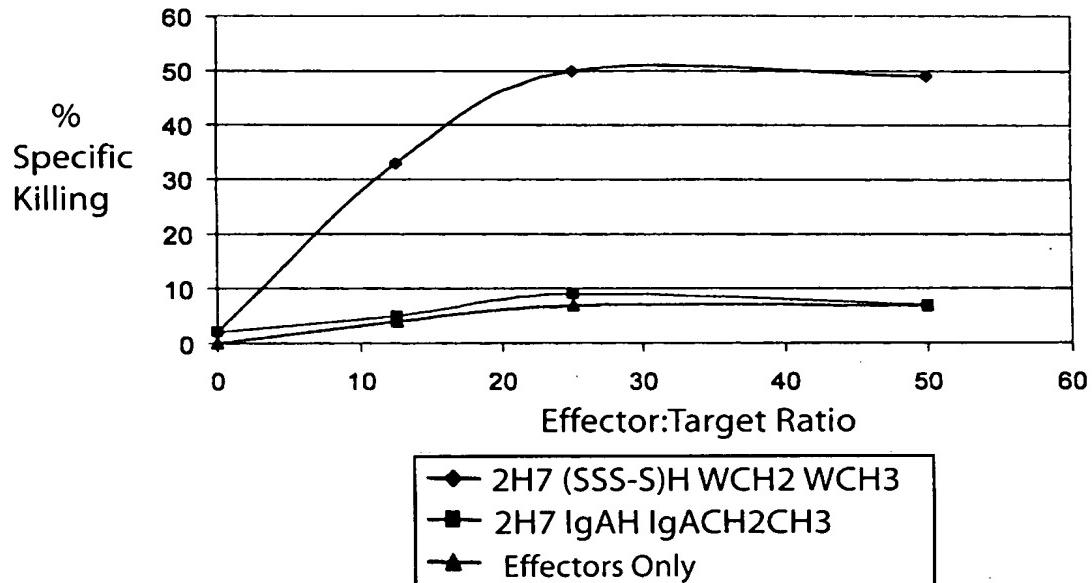
**FIG.38**

ADCC ASSAY OF ANTI-CD20 CONSTRUCTS WITH DIFFERENT TAILS  
 (WHOLE BLOOD EFFECTORS/BJAB TARGETS)

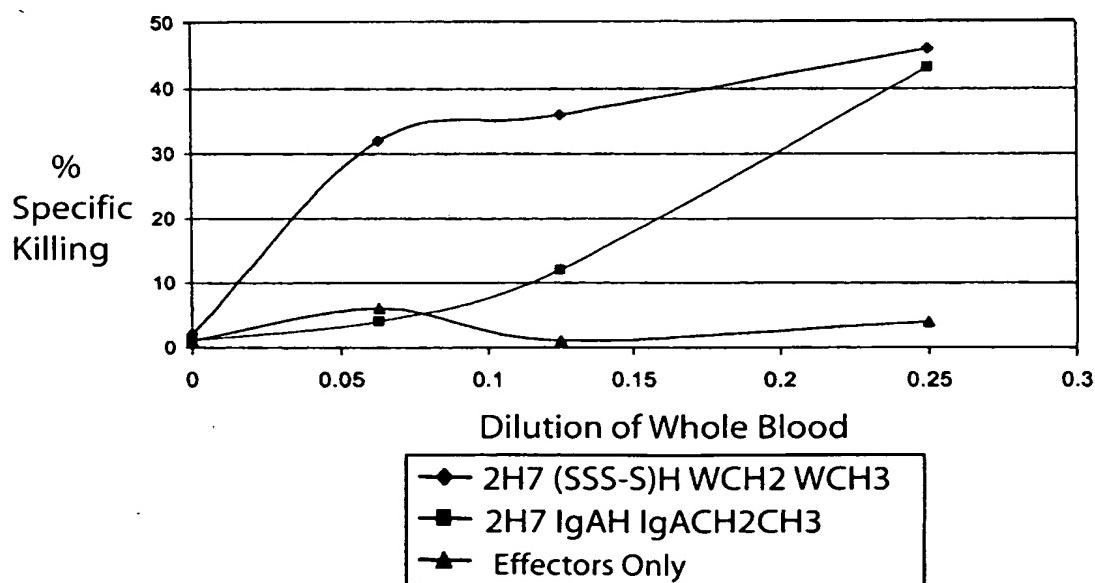


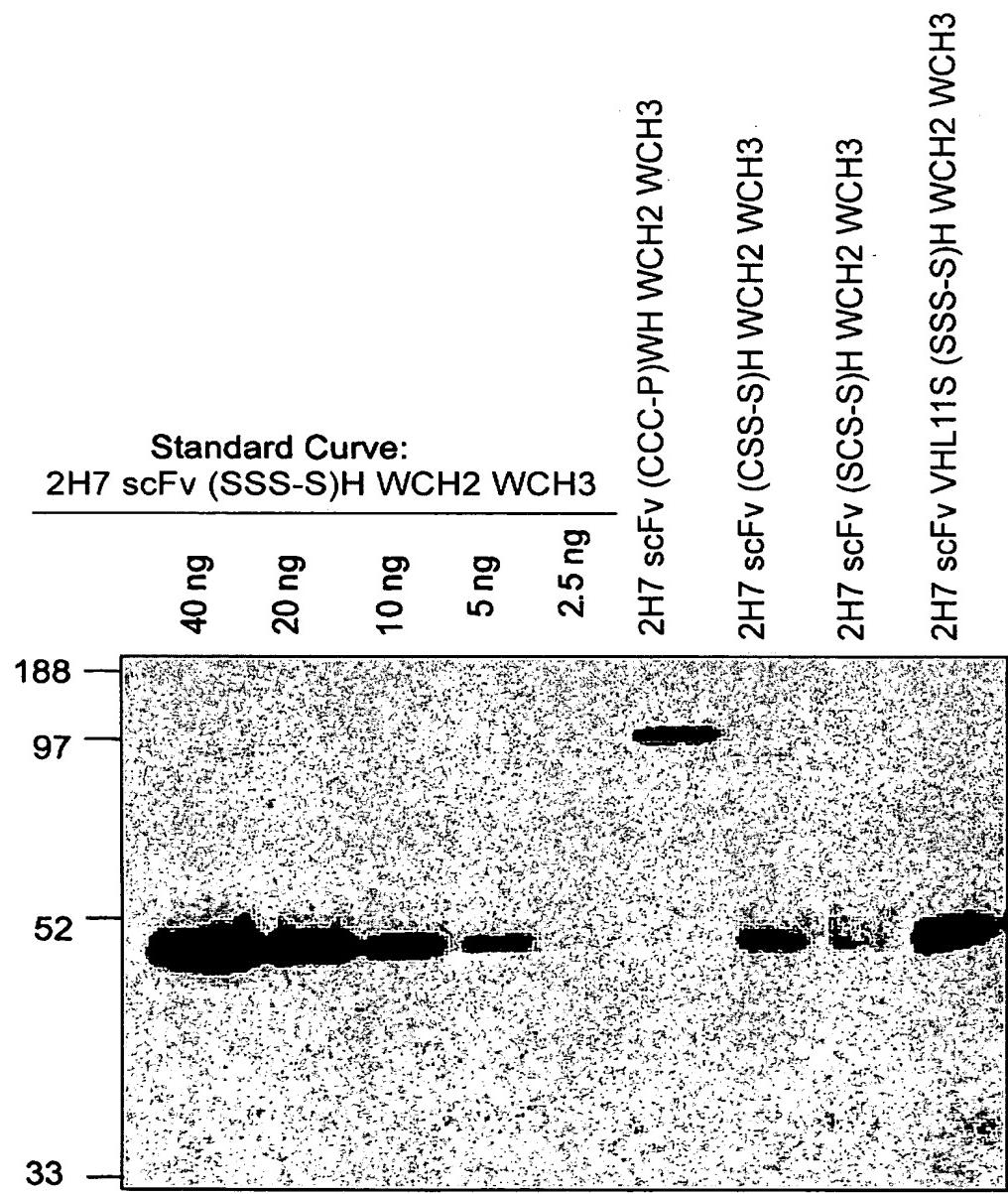
**FIG. 39A**

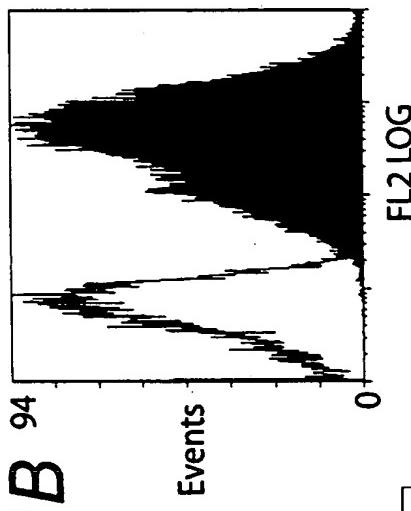
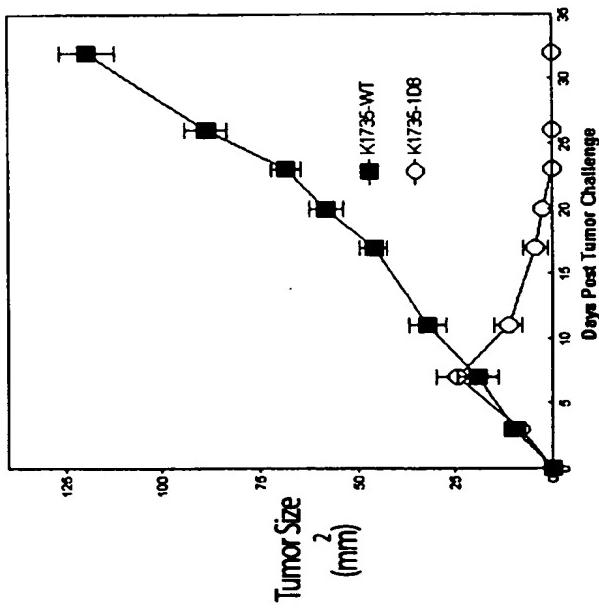
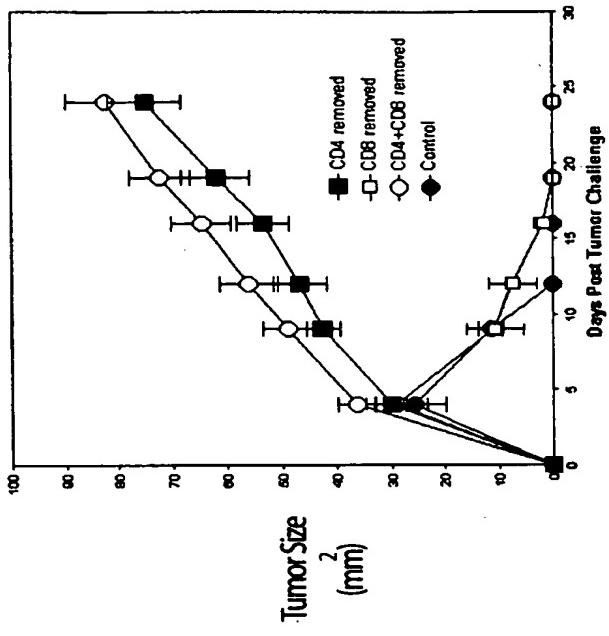
**ADCC ASSAY OF ANTI-CD20 CONSTRUCTS  
(PBMC EFFECTORS/BJAB TARGETS)**

**FIG. 39B**

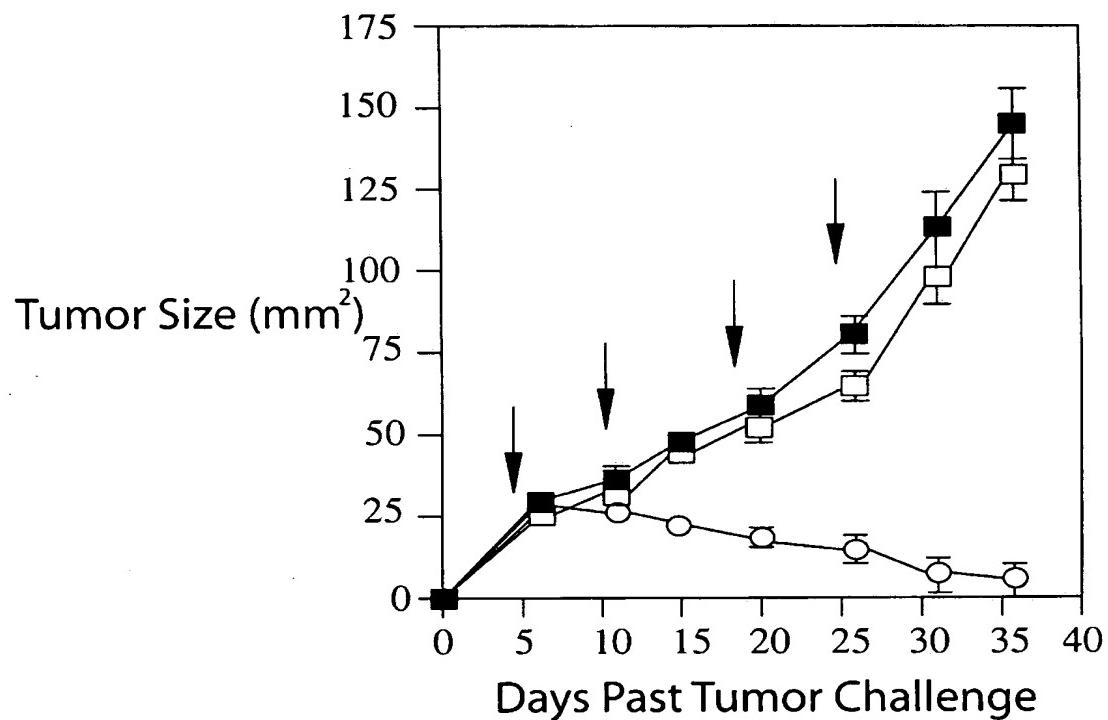
**ADCC ASSAY OF ANTI-CD20 CONSTRUCTS  
(WHOLE BLOOD EFFECTORS/BJAB TARGETS)**



**FIG. 40**

**FIG. 41B****FIG. 41C****FIG. 41D**

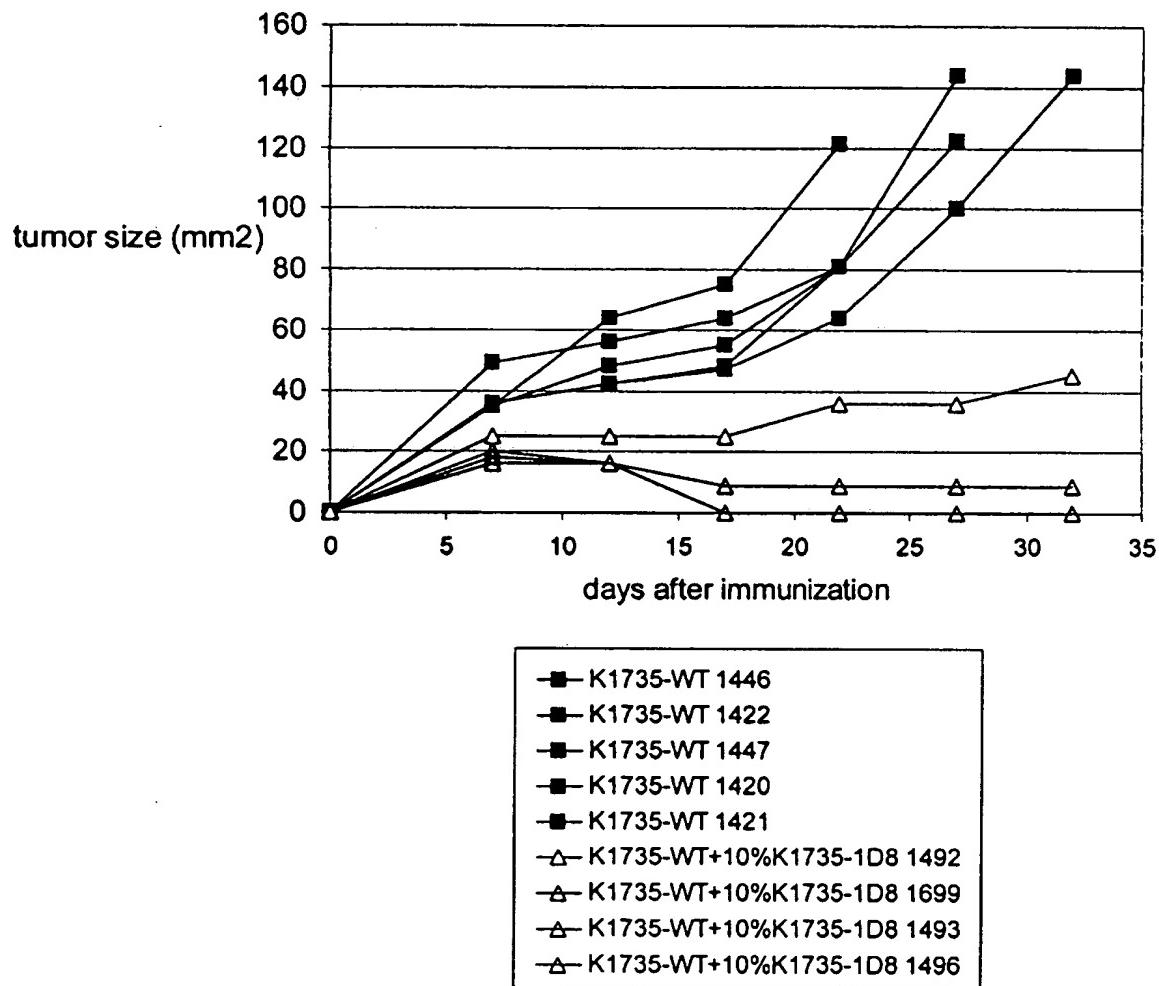
*FIG.42*



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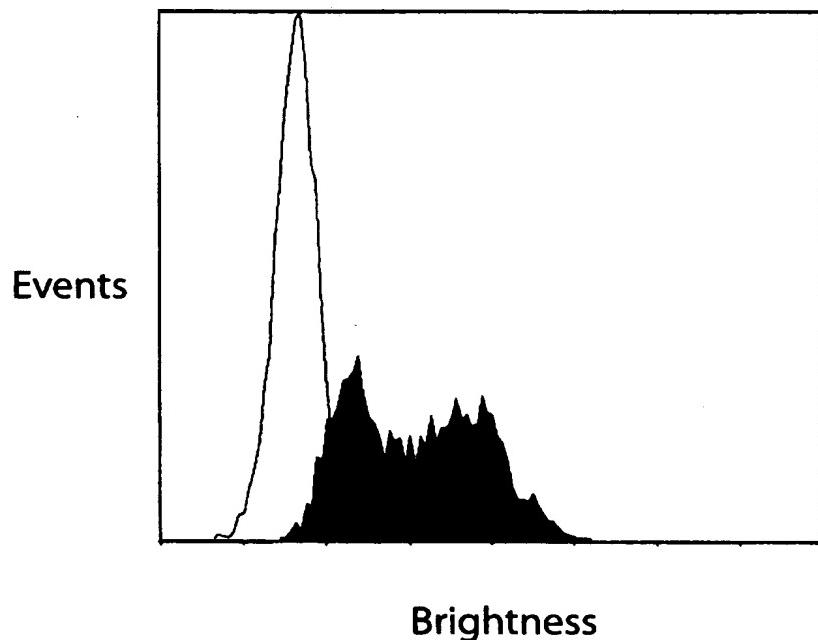
## FIG. 43

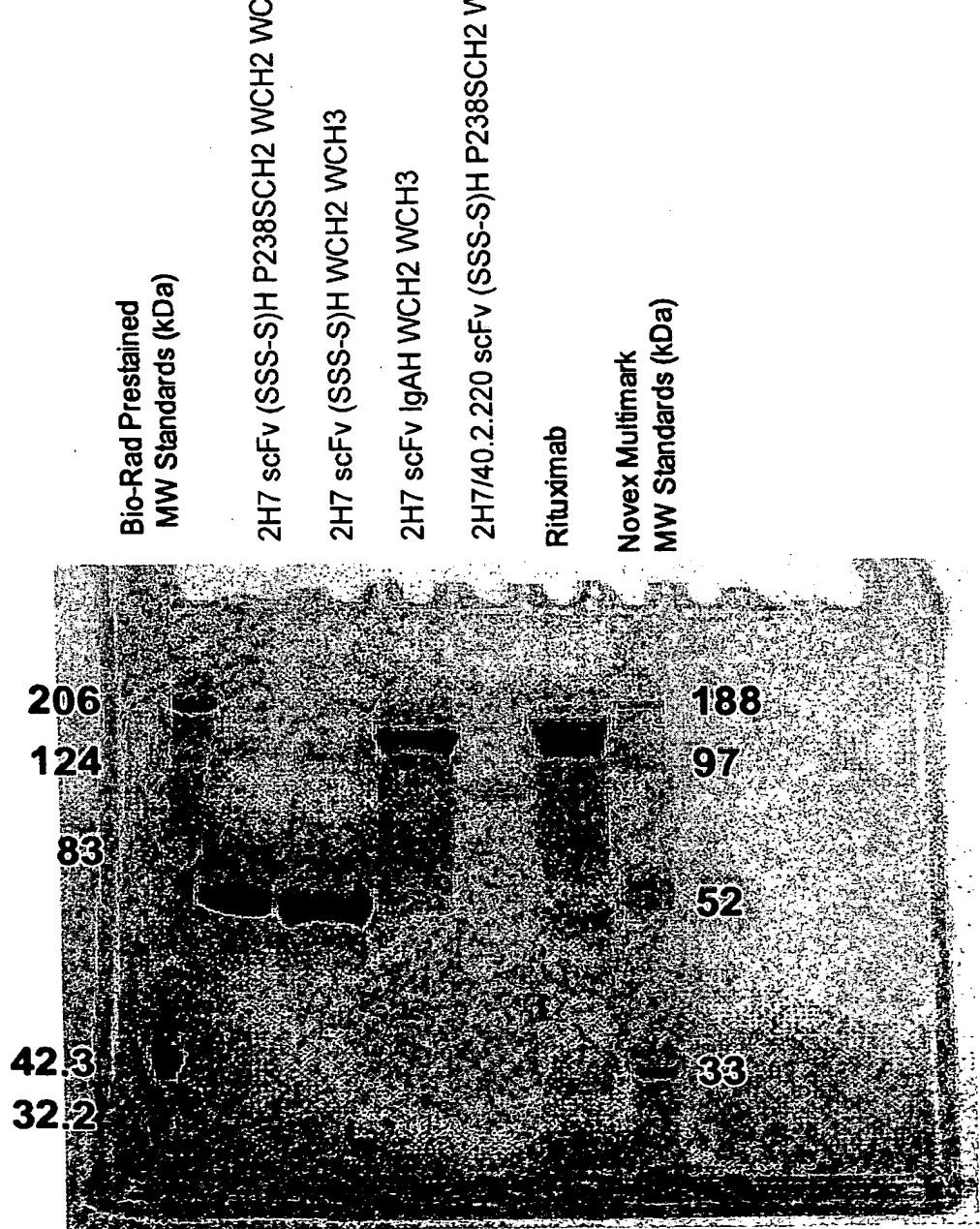
Mixtures of K1735-WT and K1735-1D8 transfected tumor lines inhibit tumor outgrowth in C3H mice



**FIG. 44**

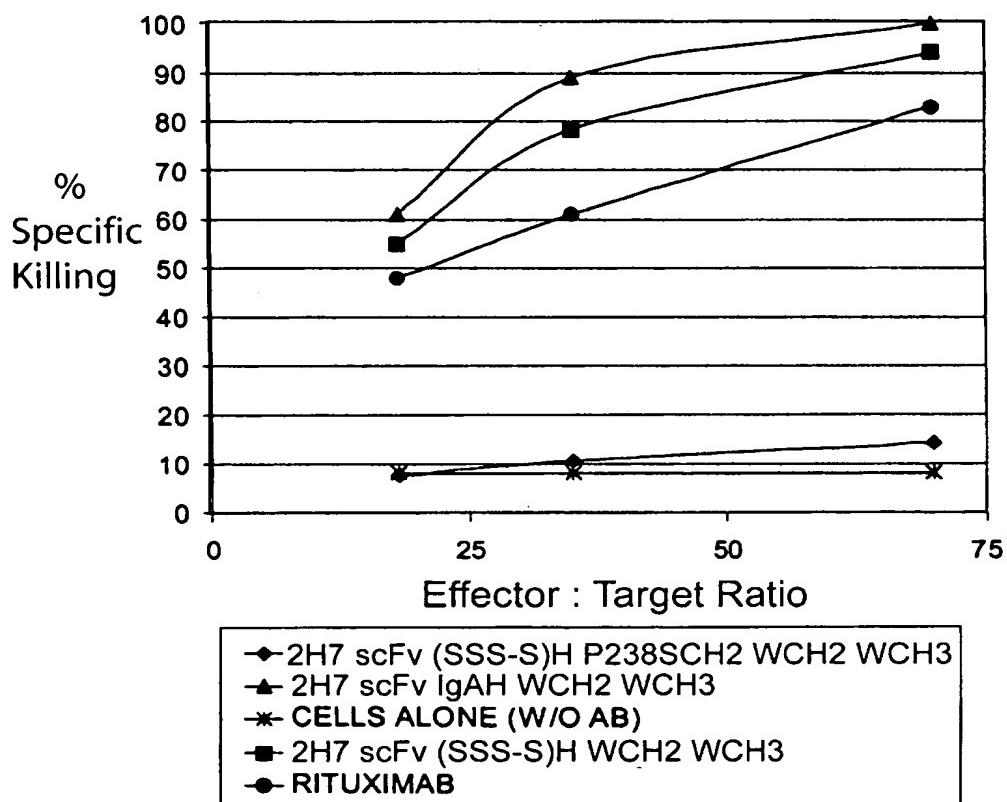
Expression of 1D8 scFv (SSS-S)H P238SCH2 WCH3 (Anti-CD37)  
on the Surface of Panned Ag104 Transfected Tumor Cells



**FIG.45**

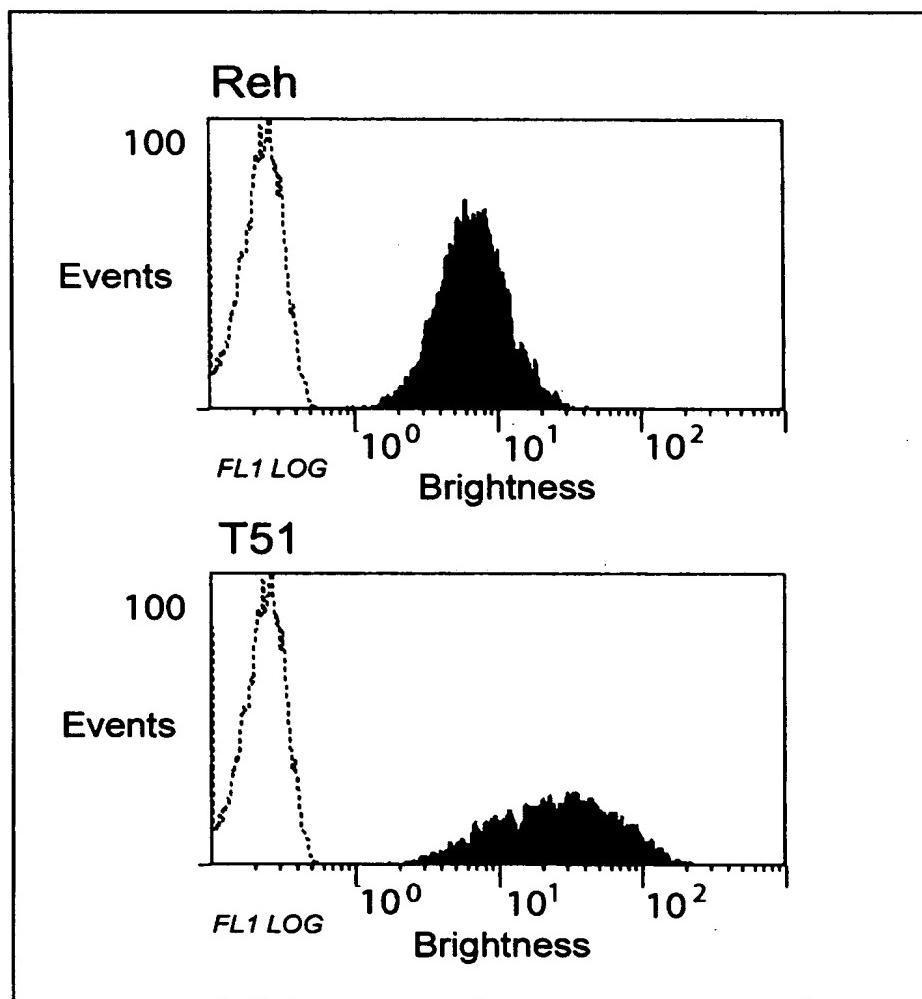
**FIG.46**

ADCC mediated by 2H7 scFvIg constructs  
by human PBMC effector cells against Bjab targets



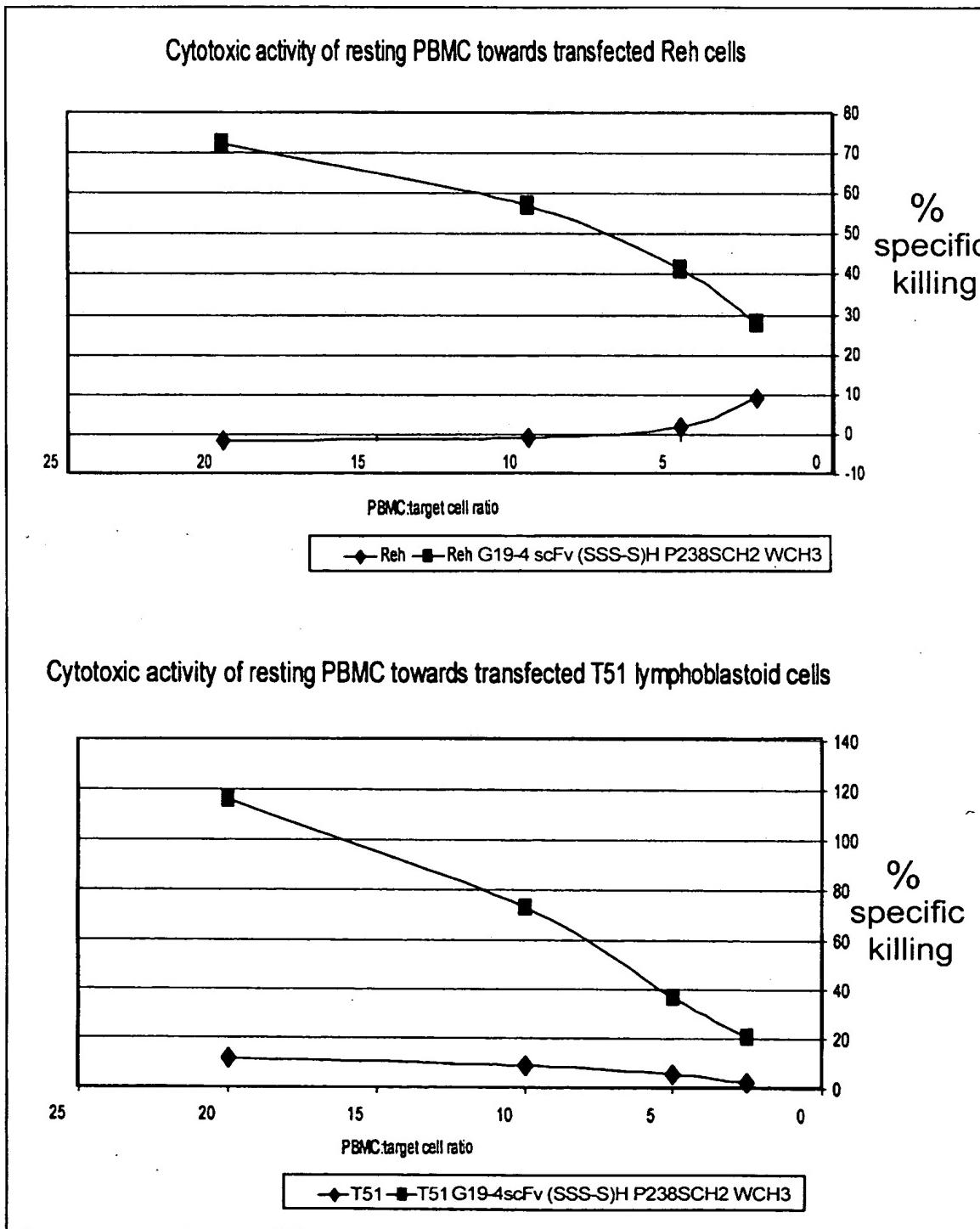
**FIG.47**

Cell surface expression of  
G19-4scFv (SSS-S)H P238SCH2 WCH3  
fusion protein on Reh and T51 Cells.



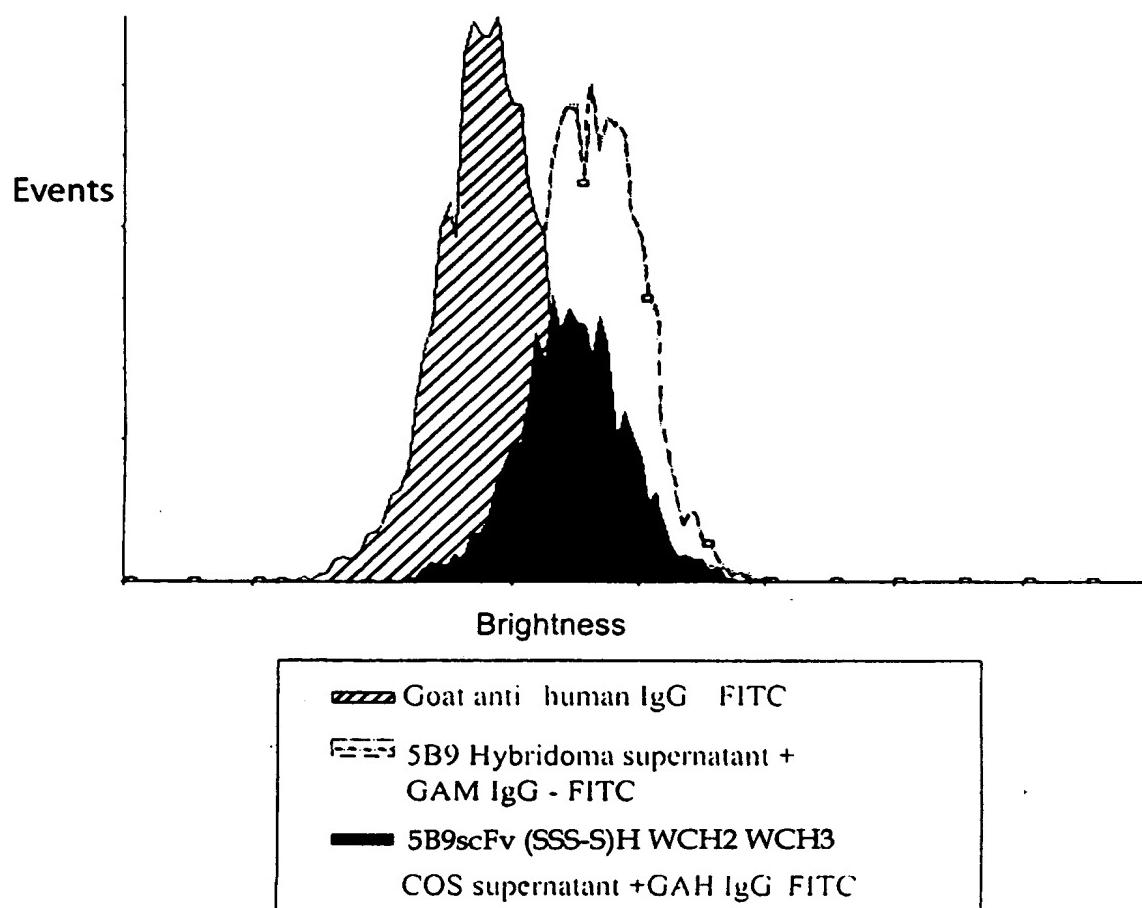
**FIG.48**

**Targeting of Cytotoxicity to Transfected Cell Lines  
by Surface expression of  
G19-4 scFv (SSS-S)H P238SCH2 WCH3**



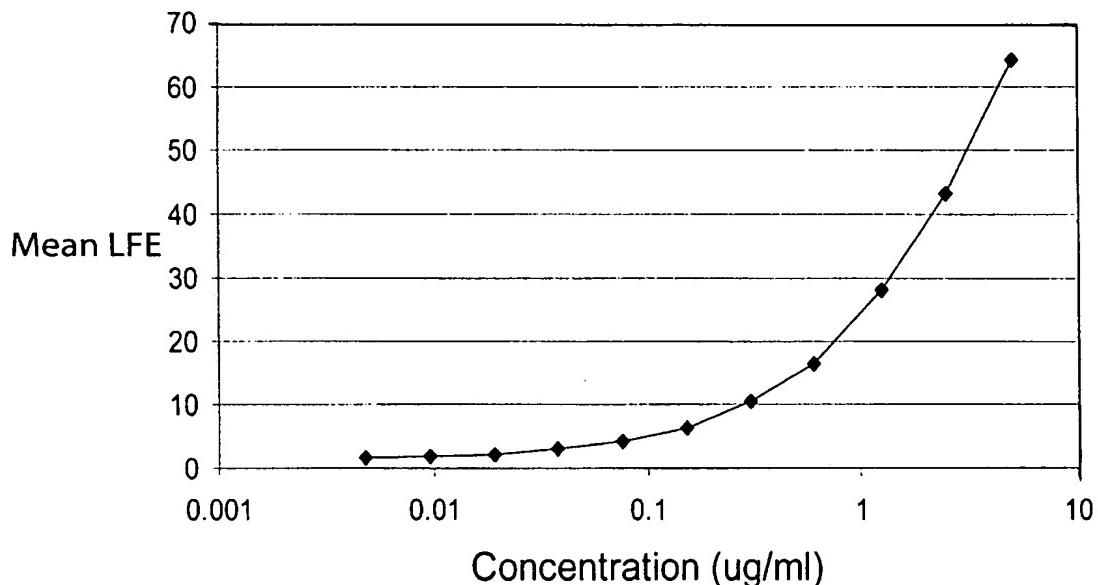
**FIG.49**

Binding of 5B9 scFv (SSS-S)H WCH2 WCH3,  
a mouse anti-human CD137 to stimulated human PBMC



**FIG.50A**

Effect of  $V_H$ L11S Mutation on  
2H7 scFv (SSS-S)H WCH2 WCH3 Protein Expression  
Standard Curve: 2H7scFv VHL11S (SSS-S)H WCH2 WCH3

**FIG.50B**

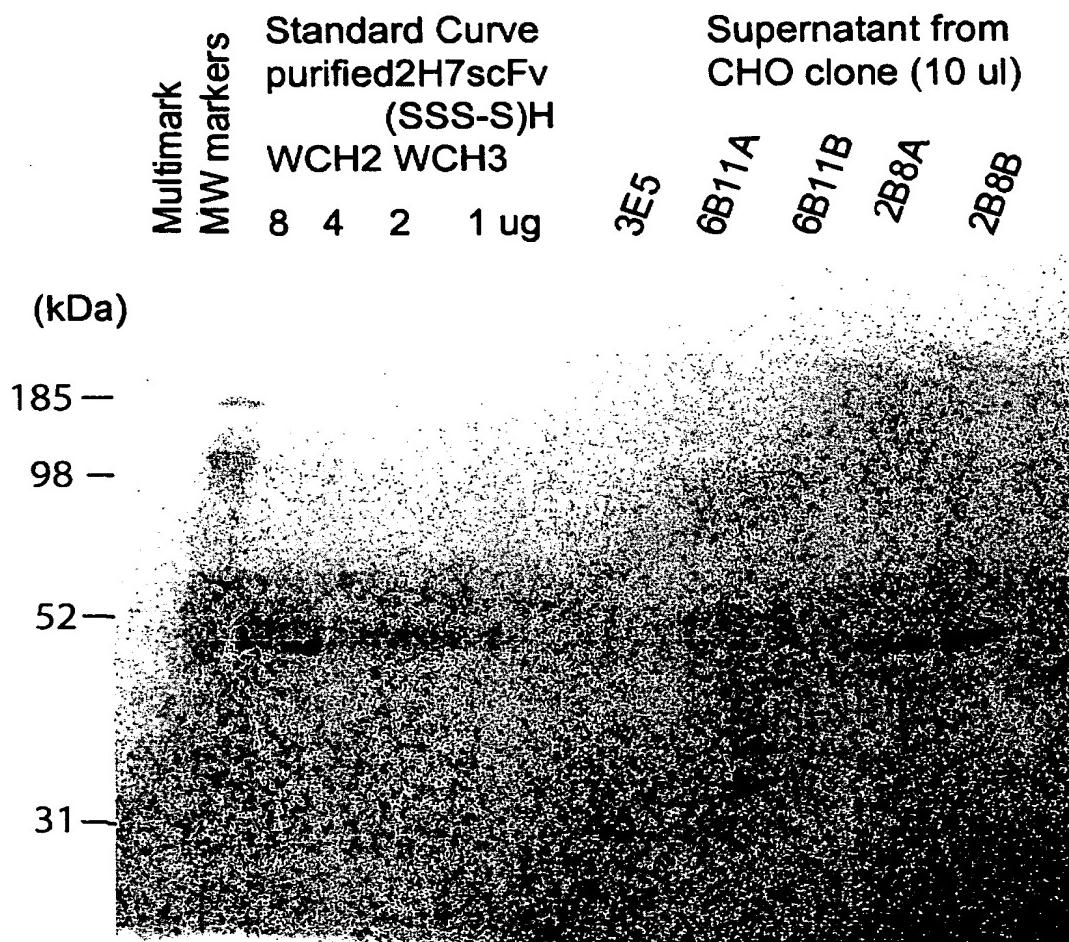
Effect of  $V_H$ L11S Mutation on  
2H7 scFv (SSS-S)H WCH2 WCH3 Protein Expression

CHO supernatant Brightness and Estimation of  
Protein concentrations from Standard Curve:

	CHO clone name				
	<u>4F2</u>	<u>4F5</u>	<u>3E5</u>	<u>6B11A</u>	<u>2B8A</u>
Mean LFE					
1/100	71.7	40.6	31.5	99.7	101.5
1/500	27.1	12.4	11.2	40.8	43
approx conc. $\mu\text{g/ml}$	600	225	125	1000	1250

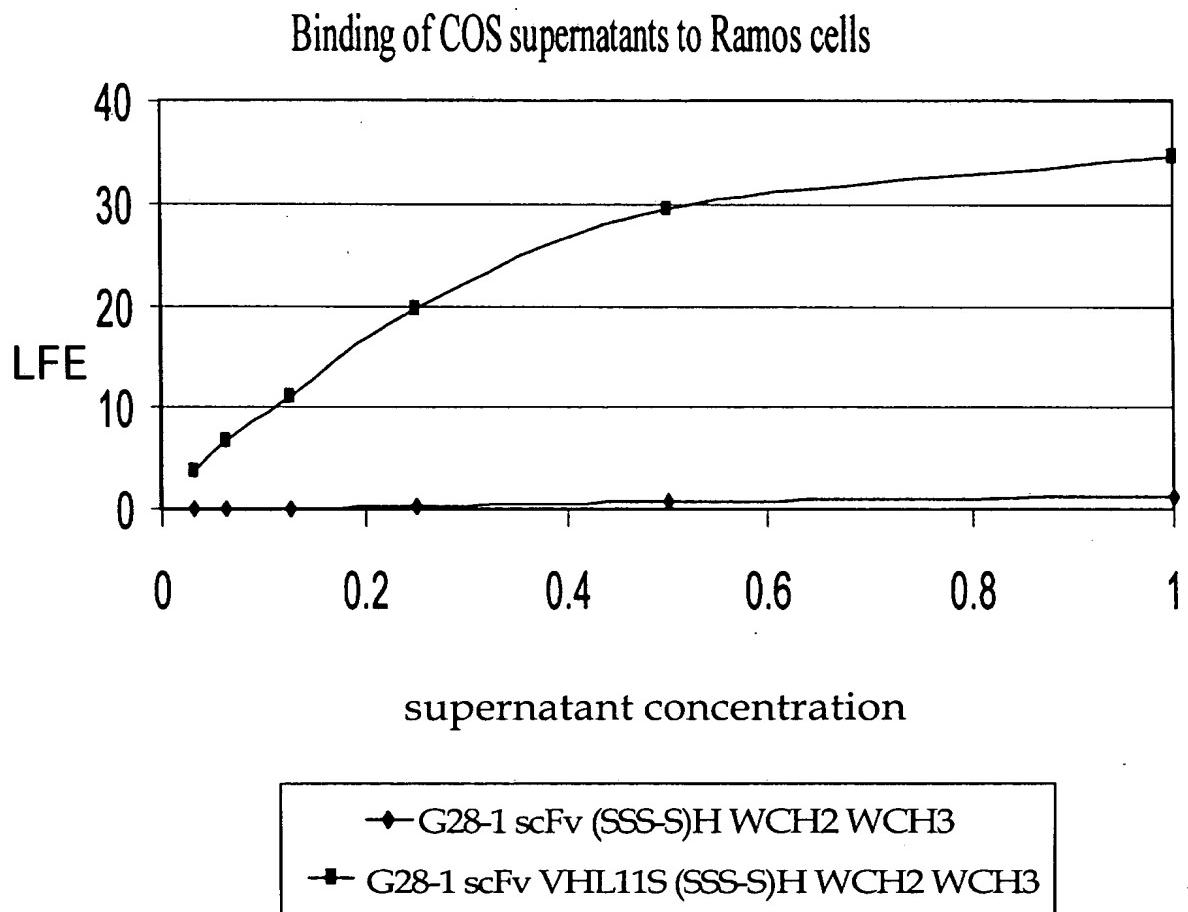
**FIG.51**

**Production Levels of 2H7scFv VH L11S  
(SSS-S)H WCH2 WCH3  
From CHO Clone Culture Supernatants**



**FIG.52**

Effect of VHL11S Mutation on G28-1 scFvIg Construct  
Protein Production from COS cells

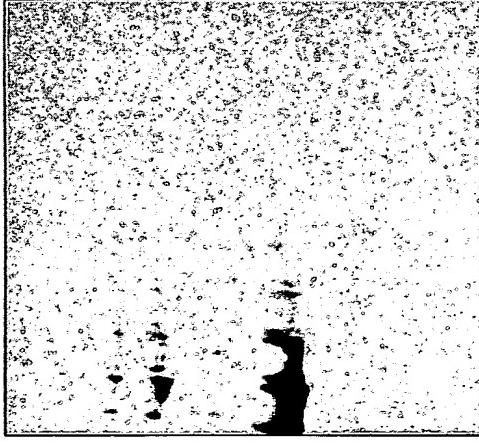


## FIG. 53A

### Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants  
transfected with G28-1scFv (SSS-S)H WCH2 WCH3  
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)

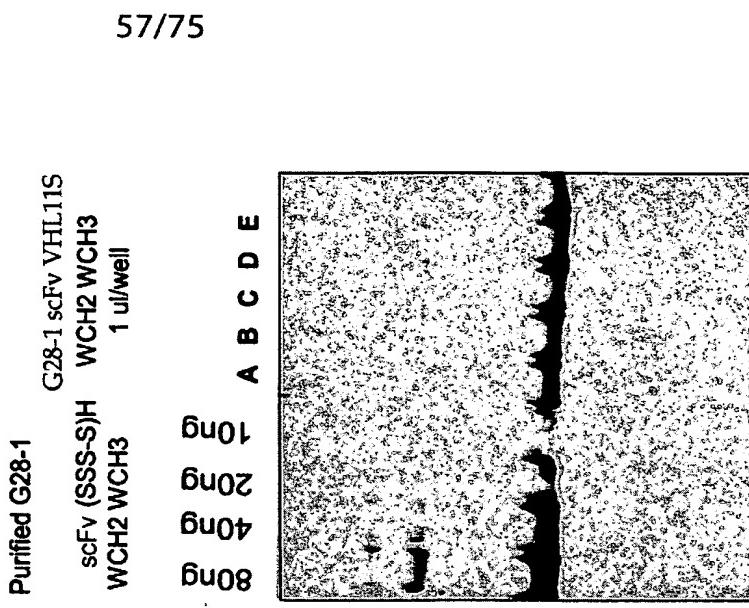
Purified G28-1	G28-1 scFv (SSS-S)H	G28-1 scFv VHL11S
scFv (SSS-S)H	WCH2 WCH3	WCH2 WCH3
WCH2 WCH3	1 $\mu$ l/well	1 $\mu$ l/well
80ng	40ng	20ng
40ng	20ng	10ng
20ng	A B C D E	A B C D E

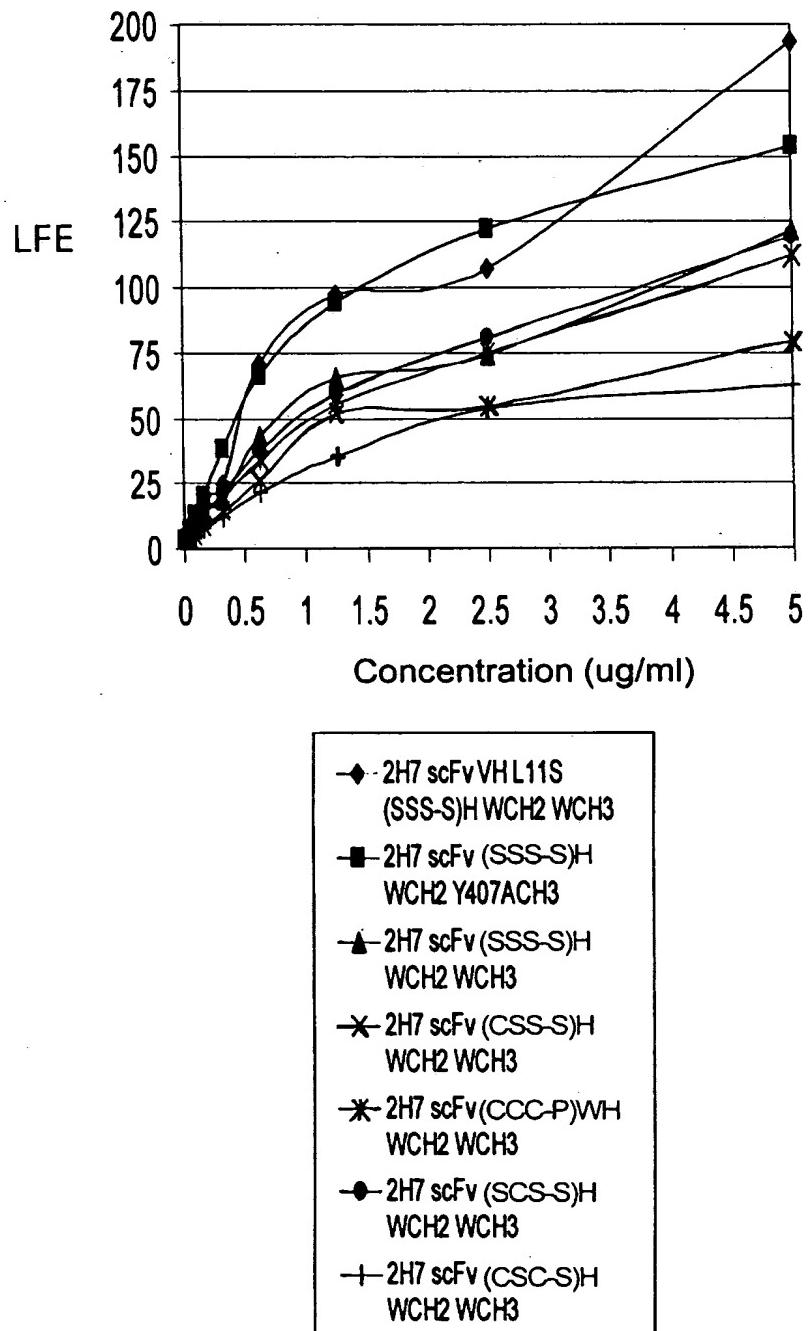


## FIG. 53B

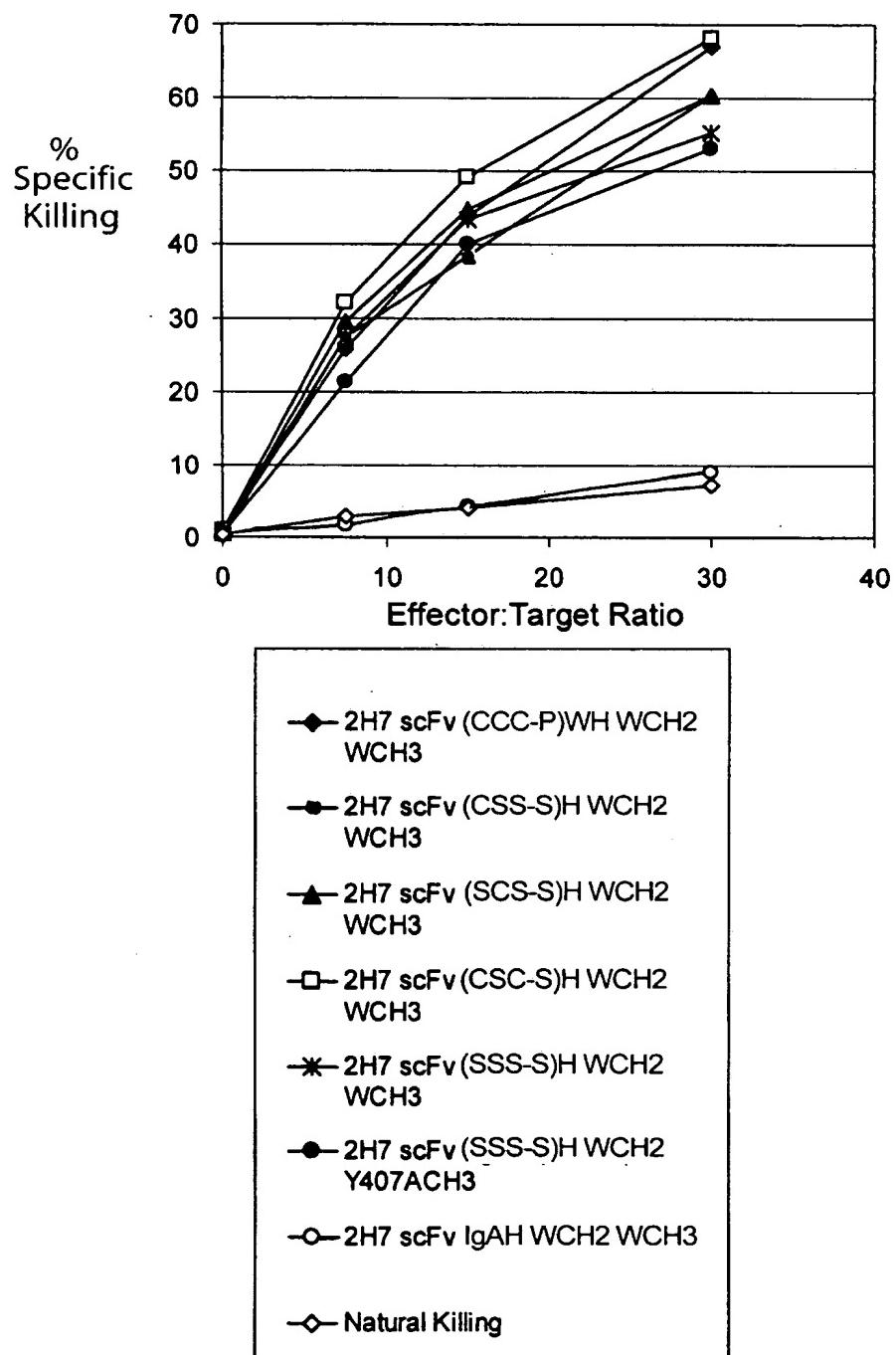
### Immunoblot of G28-1 scFvIg Constructs

Increased Protein Levels in COS supernatants  
transfected with G28-1scFv (SSS-S)H WCH2 WCH3  
After Substitution of Leucine with Serine at position 11 of VH (VHL11S)



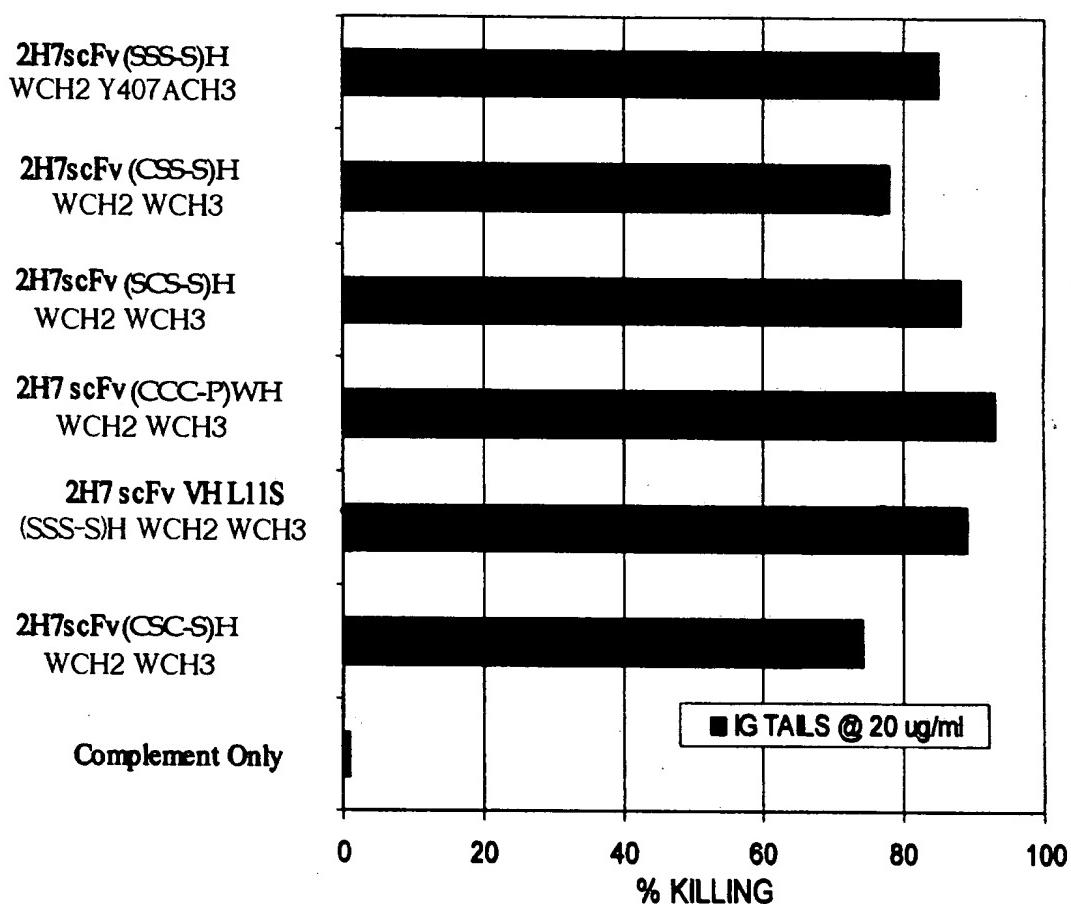
**Binding of 2H7 scFvIg Constructs with Altered Hinges and CH3 domains to CD20 CHO Cells**

ADCC Activity of 2H7 scFvlg constructs Against  
BJAB Targets and PBMC Effectors



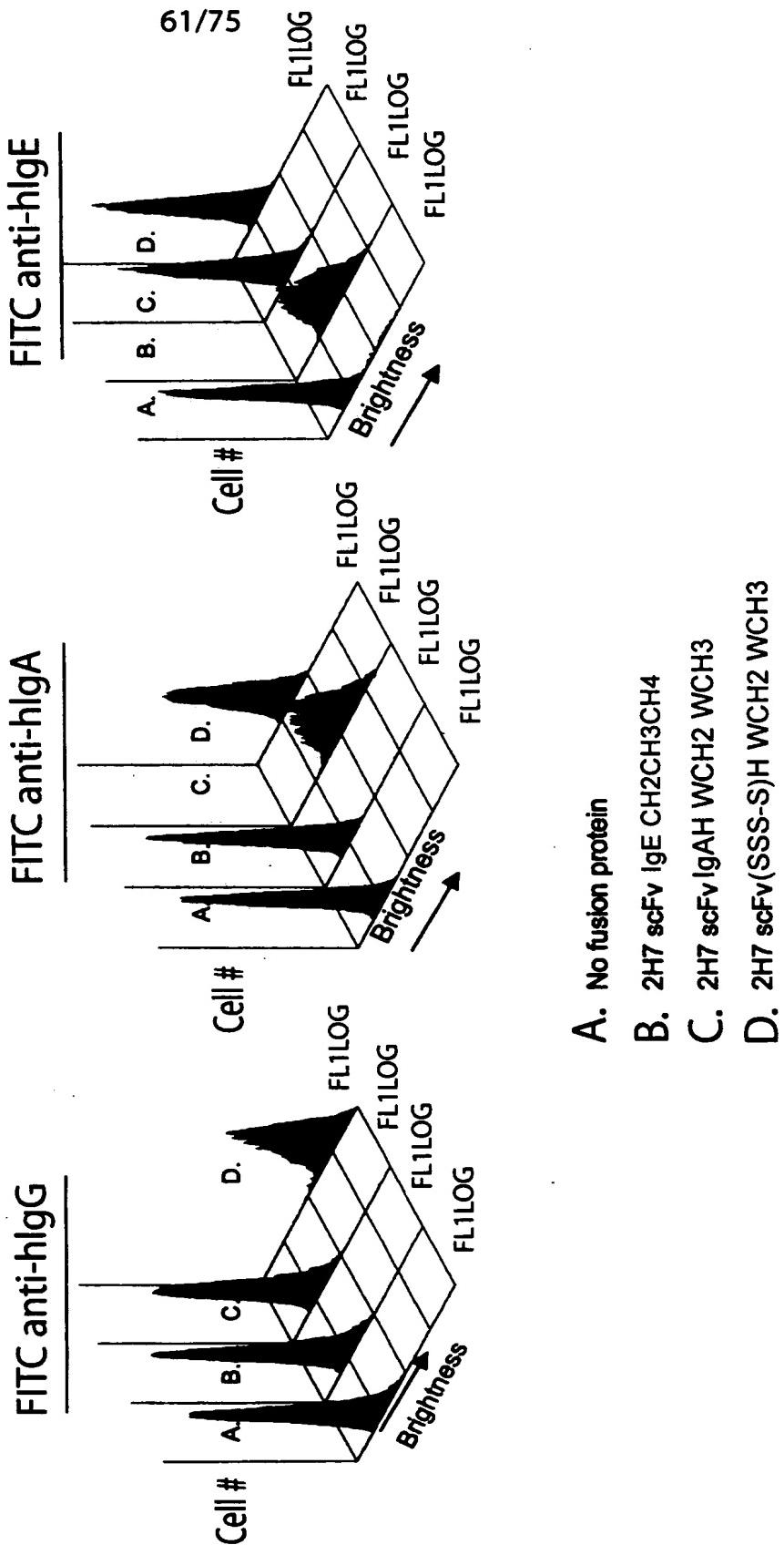
**FIG.56**

**Complement Activity of 2H7 scFv Ig Constructs  
With Ramos Target Cells**



*FIG. 57*

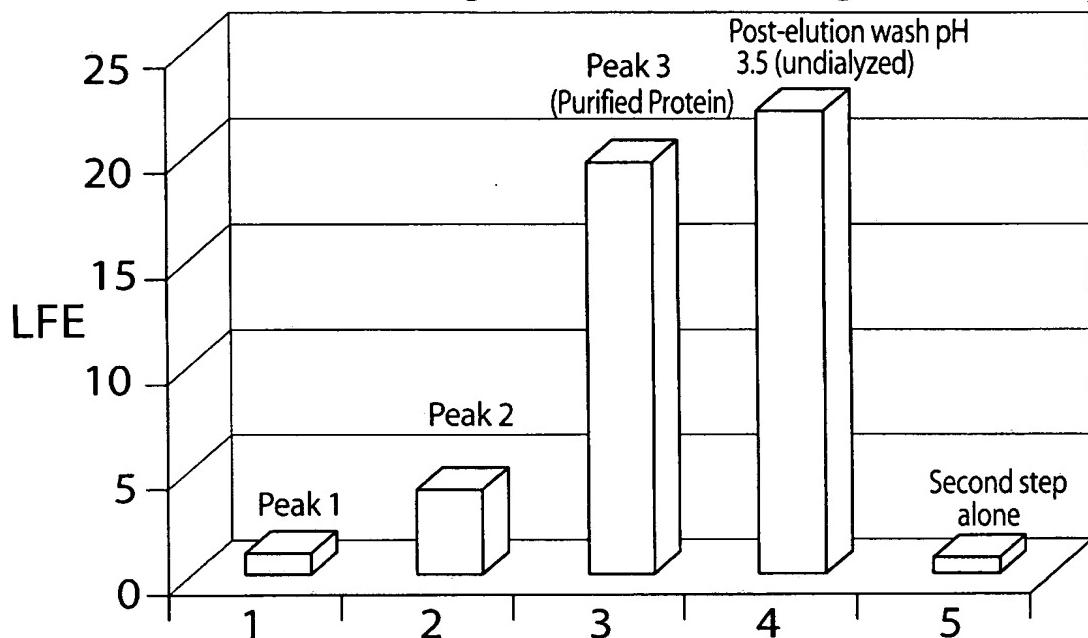
Binding of 2H7 scFvIg Derivatives CD20CHO Cells



**FIG. 58A**

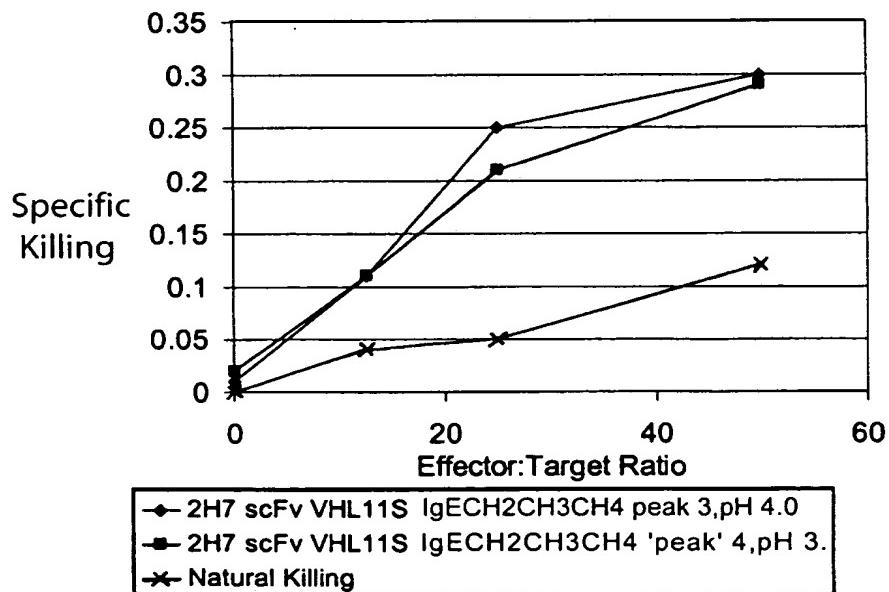
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2H7 scFv VH L11S human IgE CH₂CH₃CH₄  
Binding to CD20 CHO at 30 ug/ml



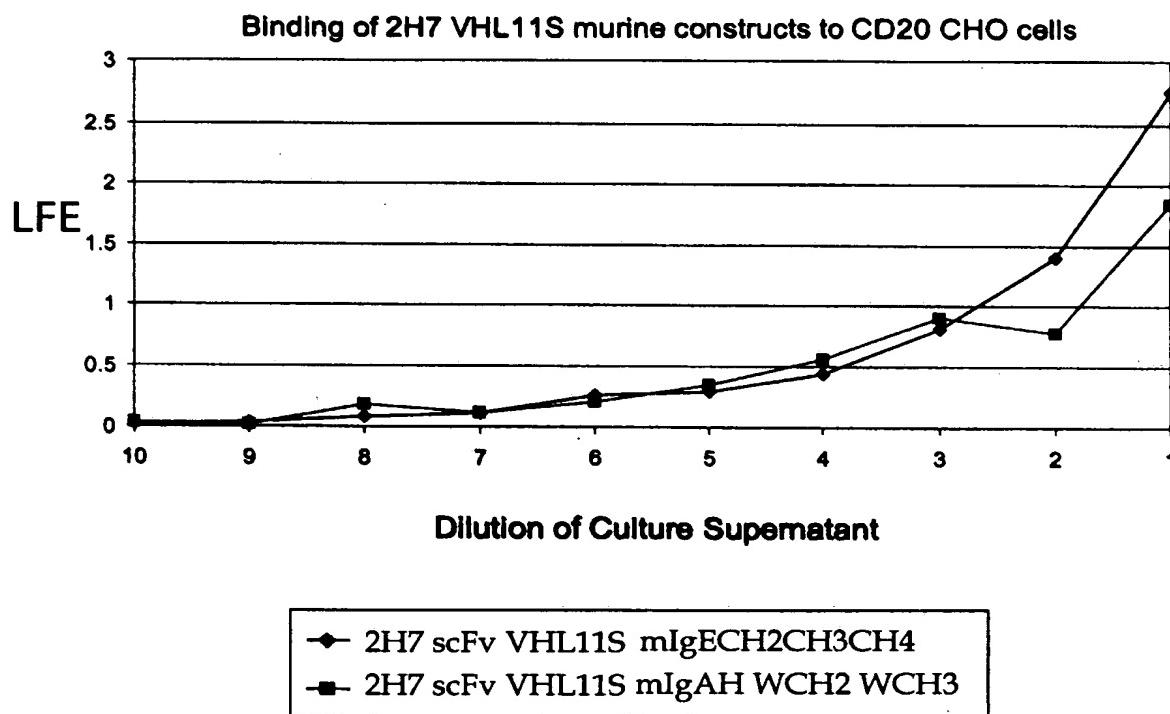
**FIG. 58B**

ADCC Activity of 2H7 scFv VHL11S IgE CH₂CH₃CH₄  
Protein Fractions with PBMC Effectors and Bjab Targets

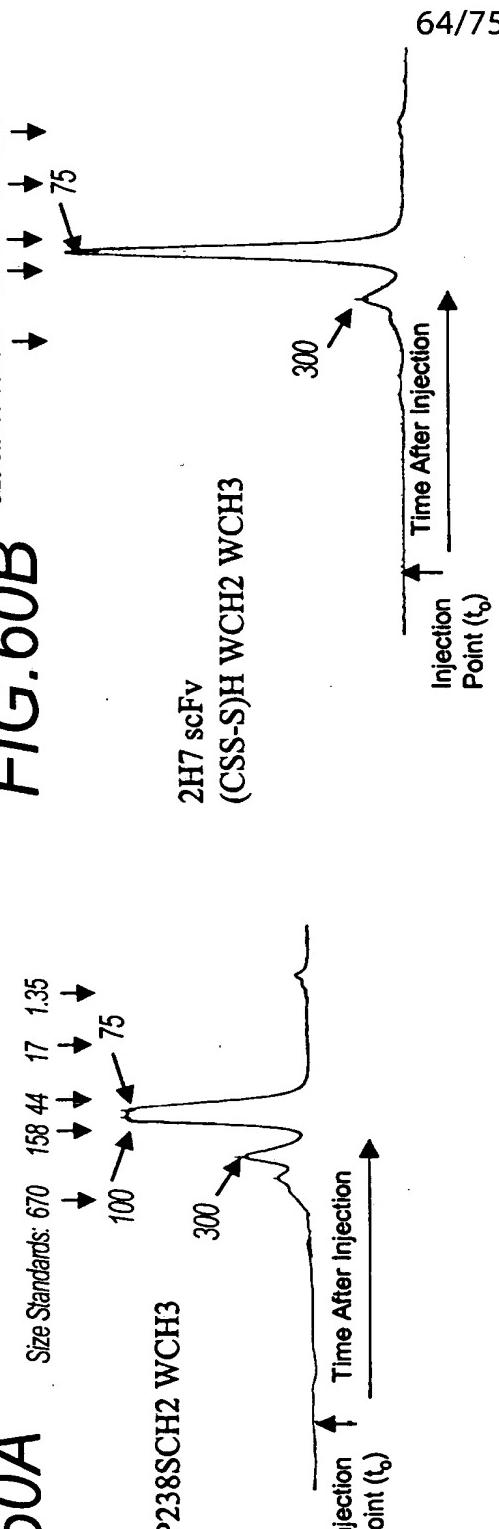


**FIG. 59**

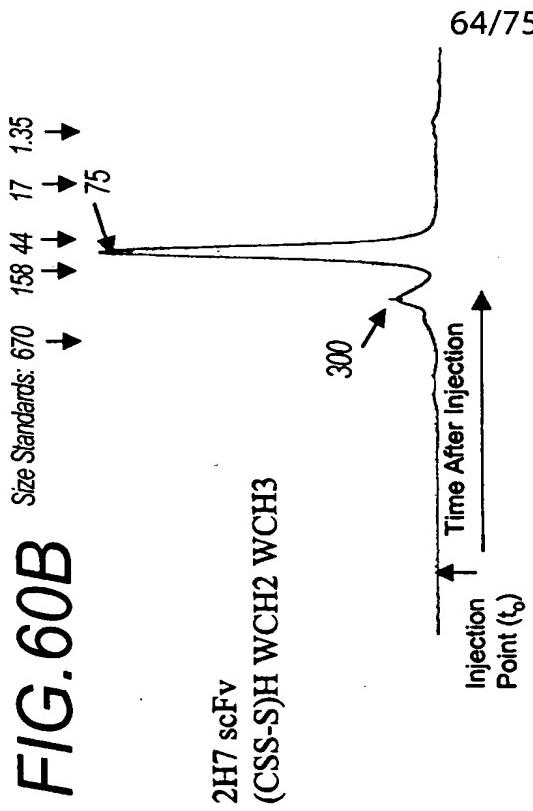
Binding Data For COS derived 2H7 scFv VHL11S  
mIgECH2CH3CH4 and mIgAH WCH2 WCH3



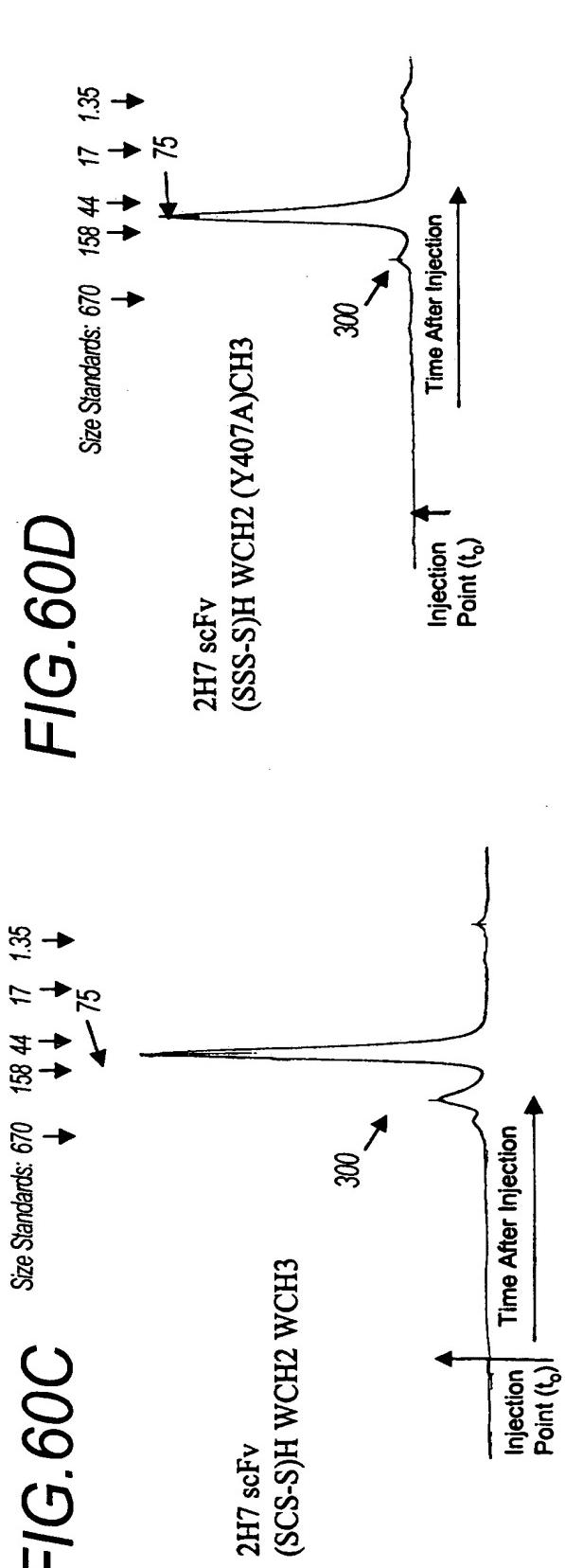
*FIG. 60A*



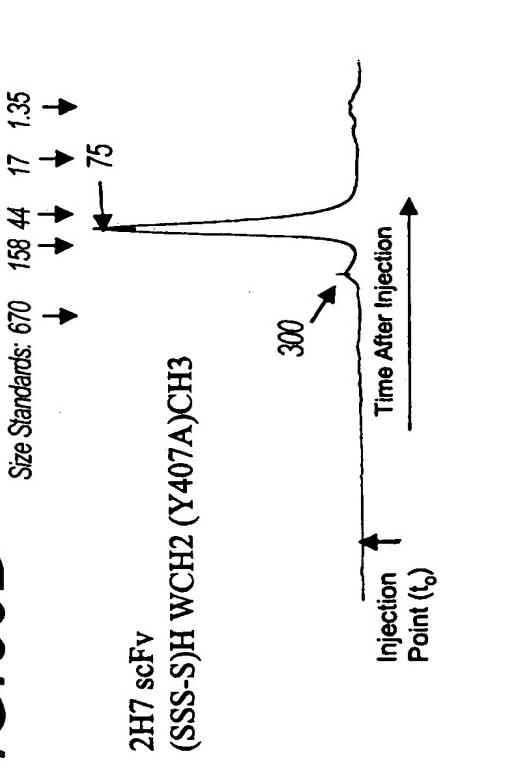
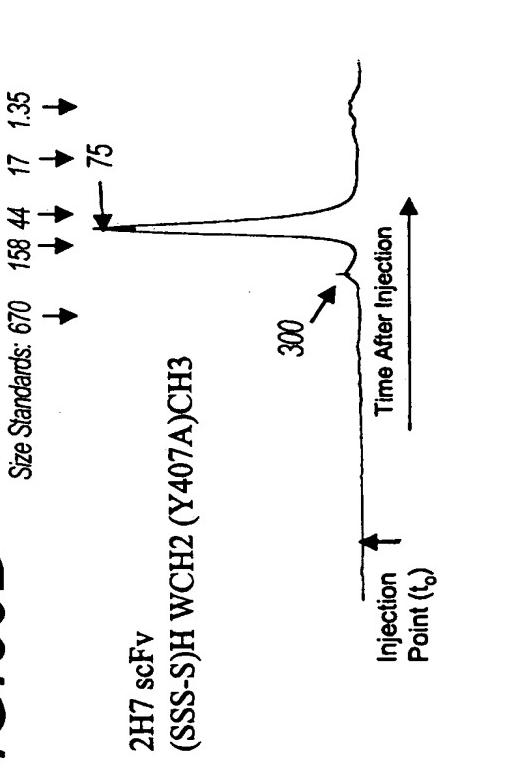
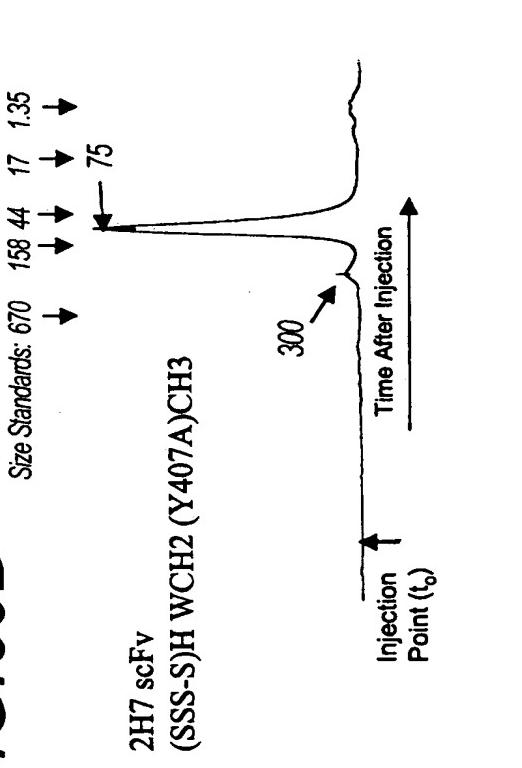
*FIG. 60B*



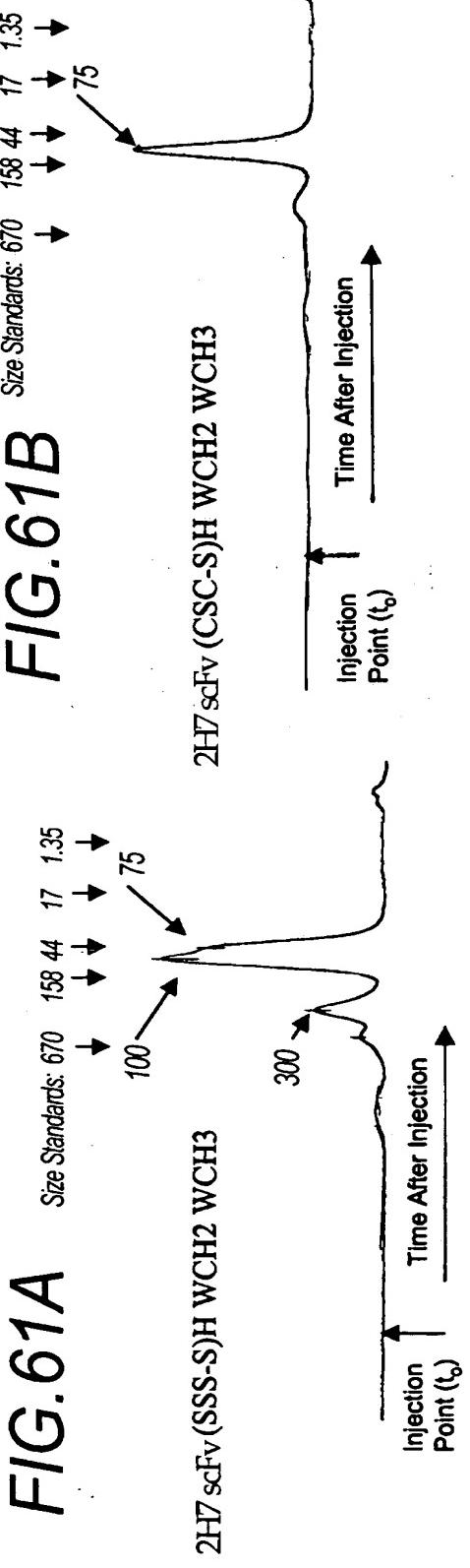
*FIG. 60C*



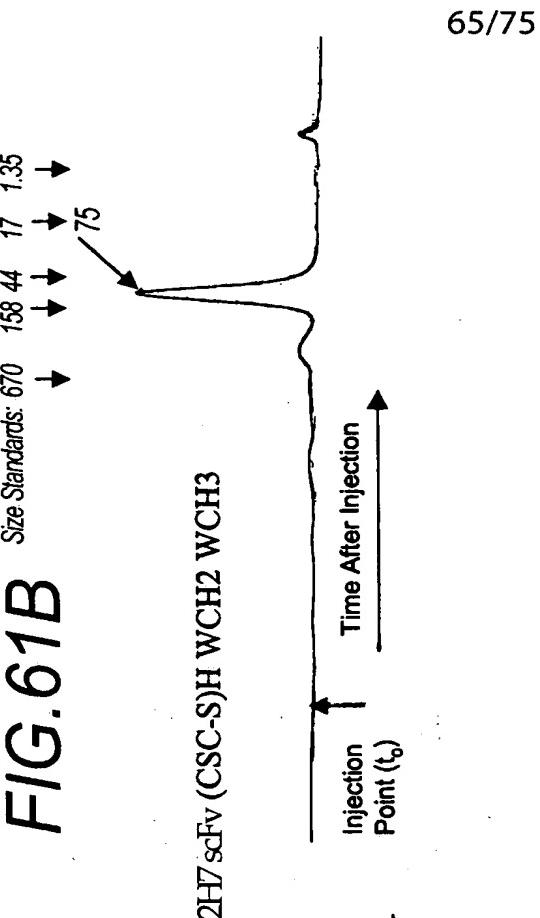
*FIG. 60D*



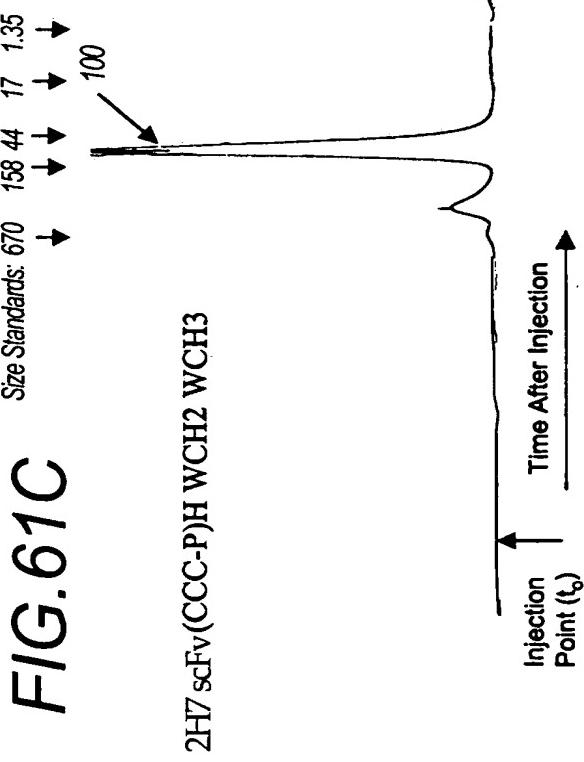
*FIG. 61A*



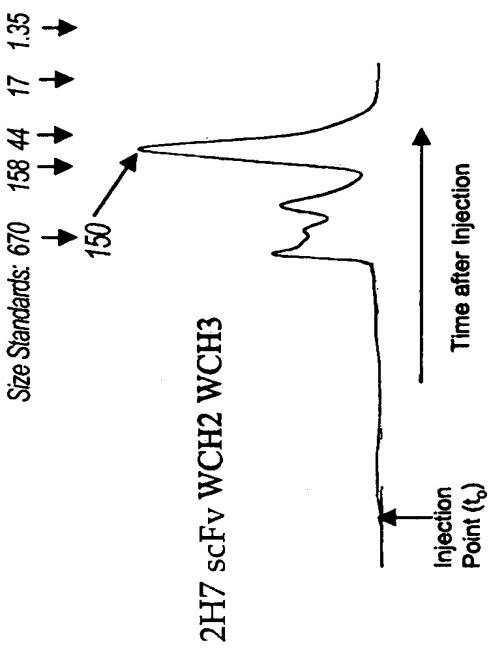
*FIG. 61B*



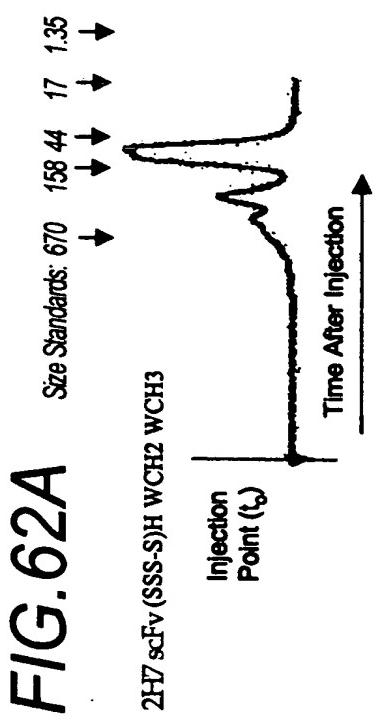
*FIG. 61C*



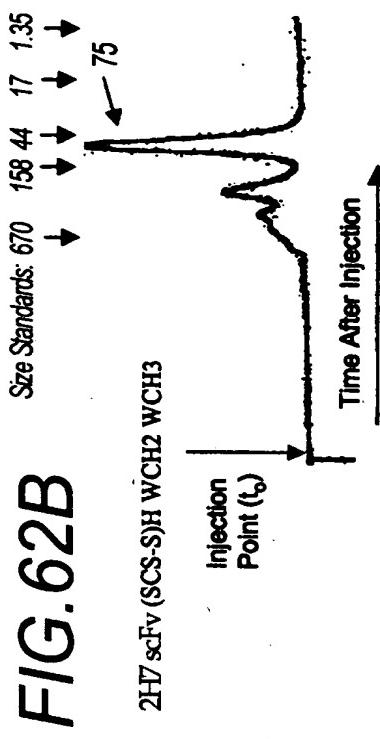
*FIG. 61D*



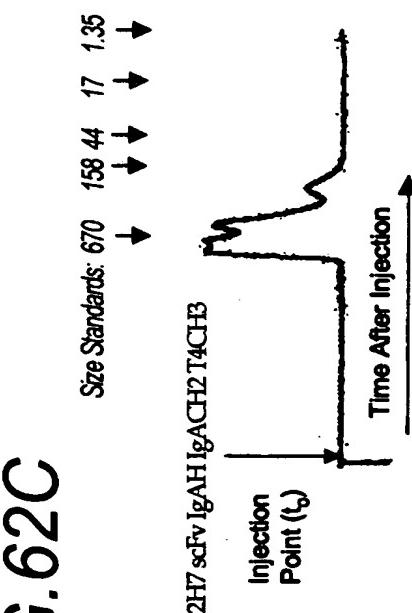
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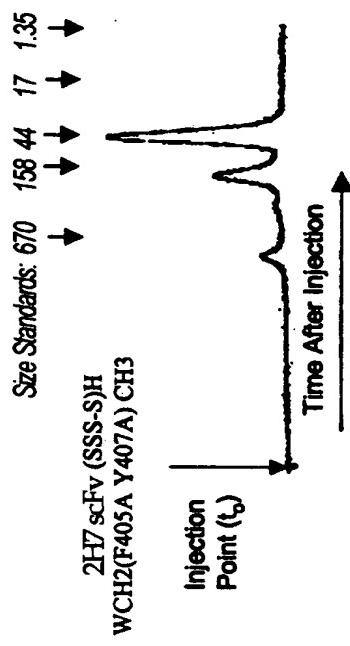
**FIG. 62B**



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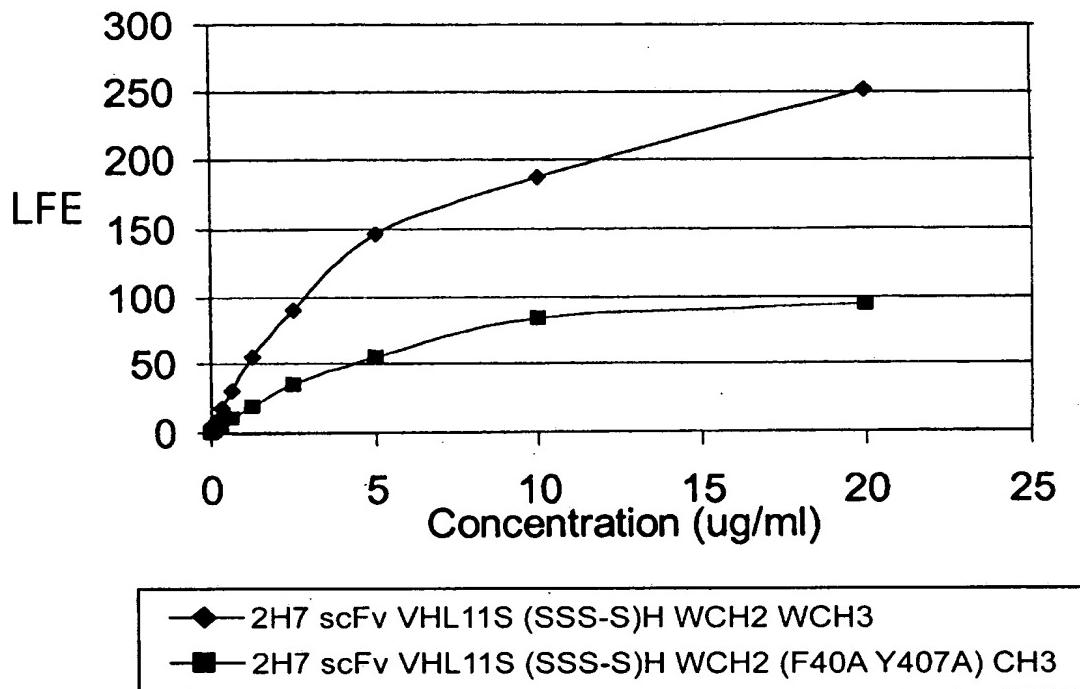


**FIG. 62D**



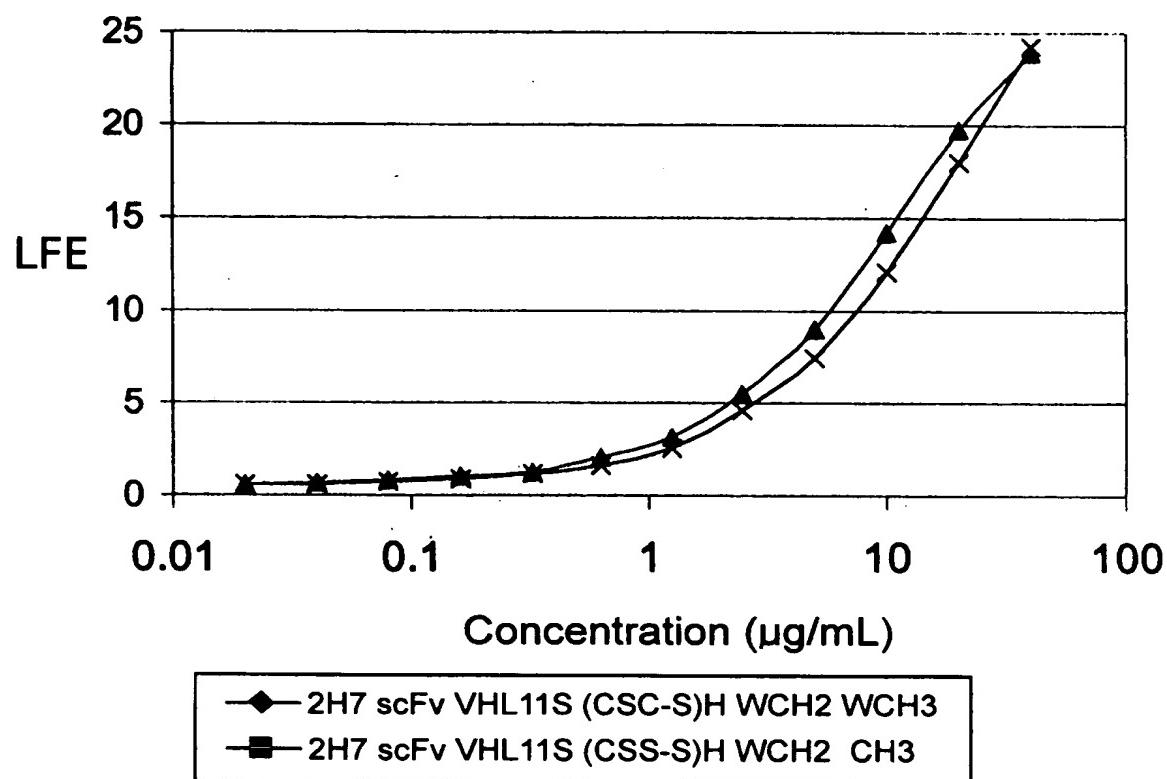
**FIG. 63**

Binding of Purified Proteins from COS Supernatants  
to CD20 CHO cells:  
Differential Effects of CH3 Mutations on Binding



**FIG.64**

Binding of FITC conjugated 2H7 scFv VHL11S Proteins to CD20 CH0 Cells



**FIG. 65**

Nonreducing SDS-PAGE on Protein A-Purified Lots  
of 2H7 scFv VHL11S Constructs (10 ug/lane)

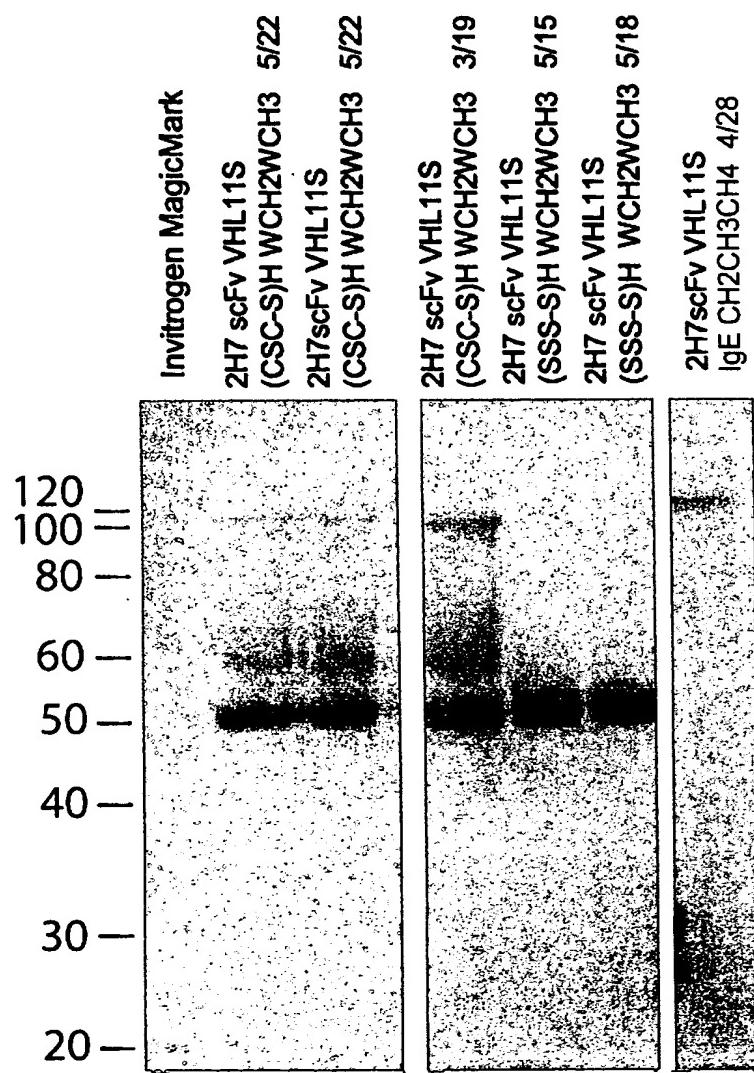
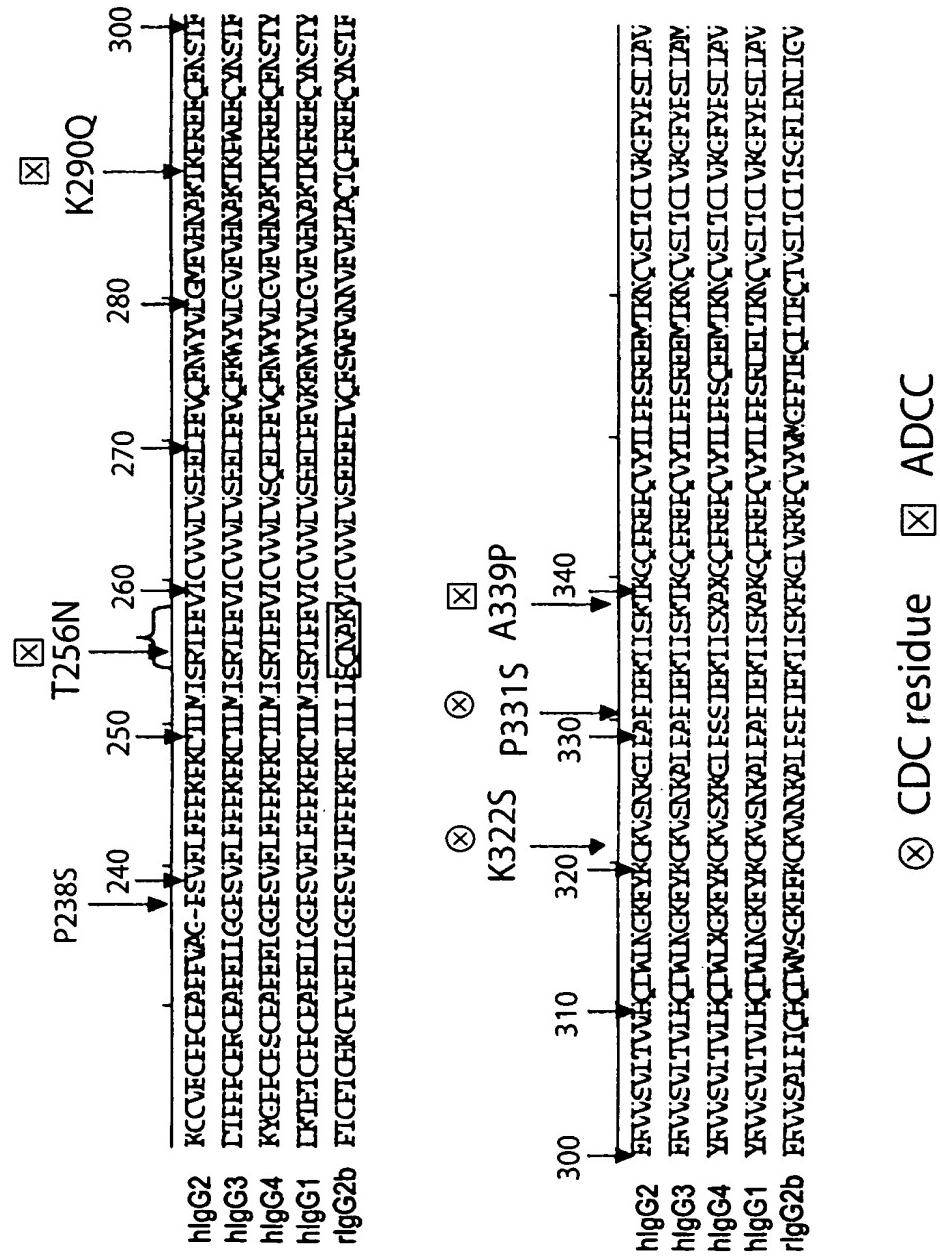


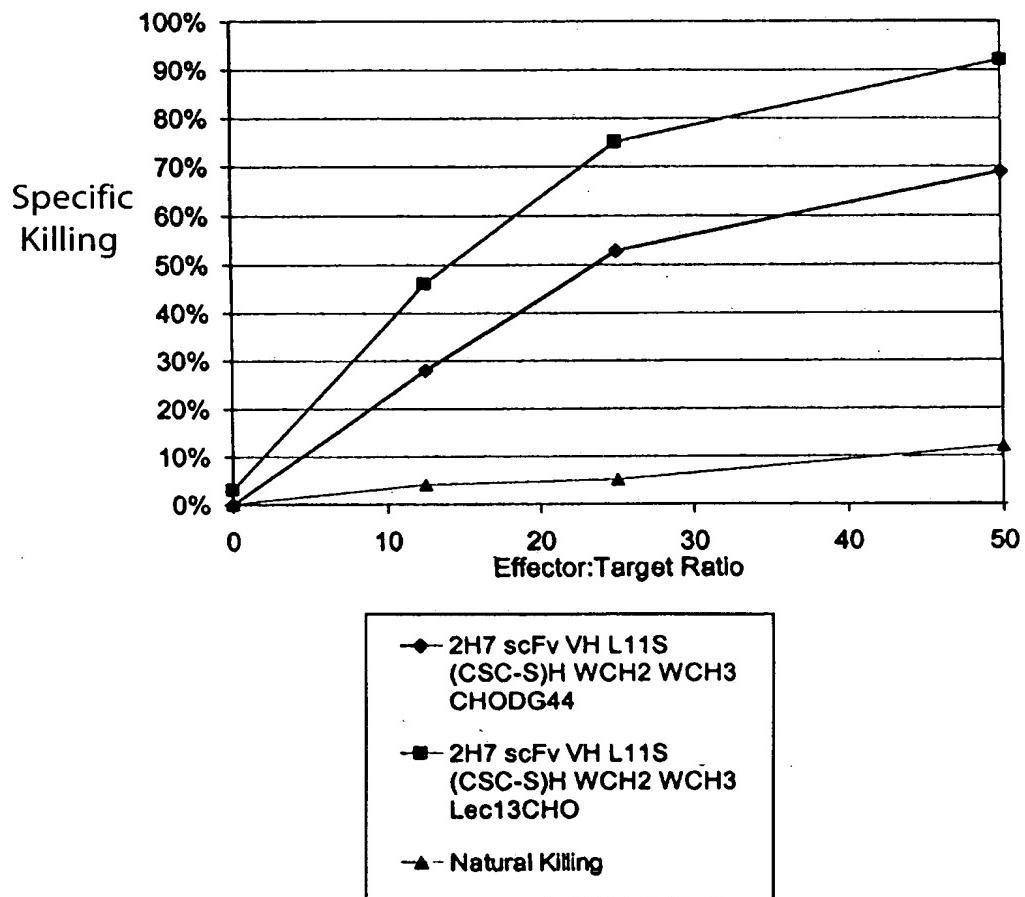
FIG. 66

## Alterations in Human IgG Fc sequence that differentially change effector function efficiency



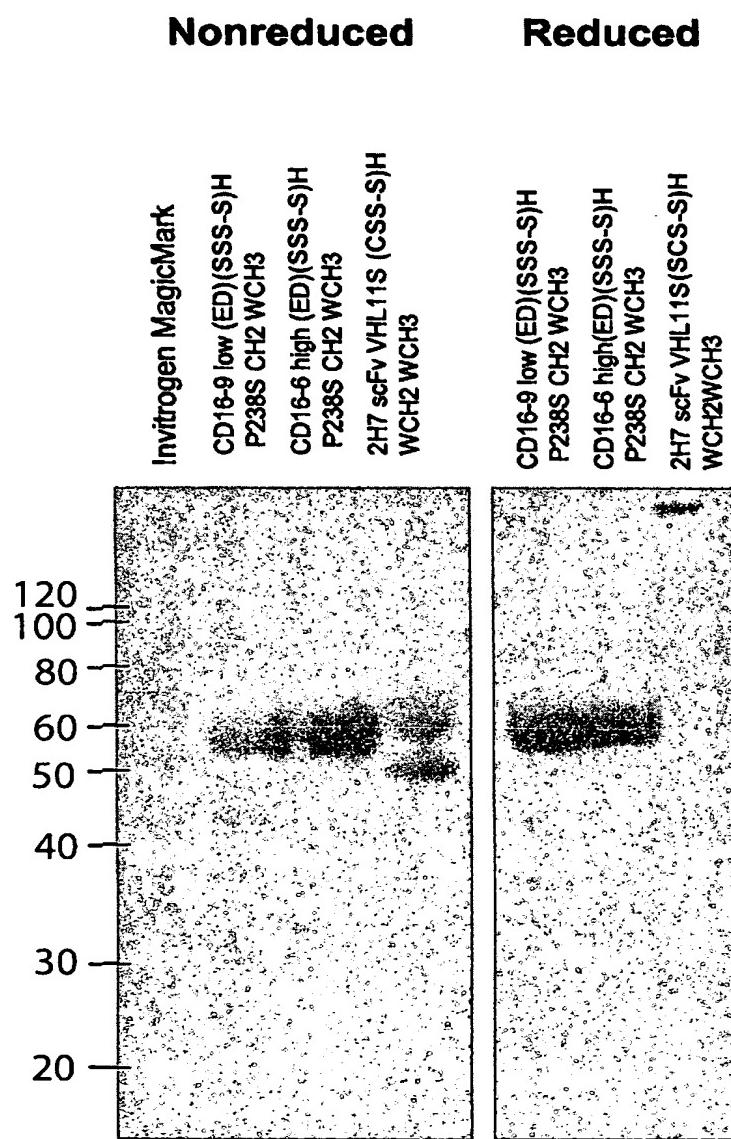
**FIG. 67**

ADCC Activity of 2H7 scFv VHL11S (CSC-S)H WCH2 WCH3 from CHO and Lec13-CHO transient transfections



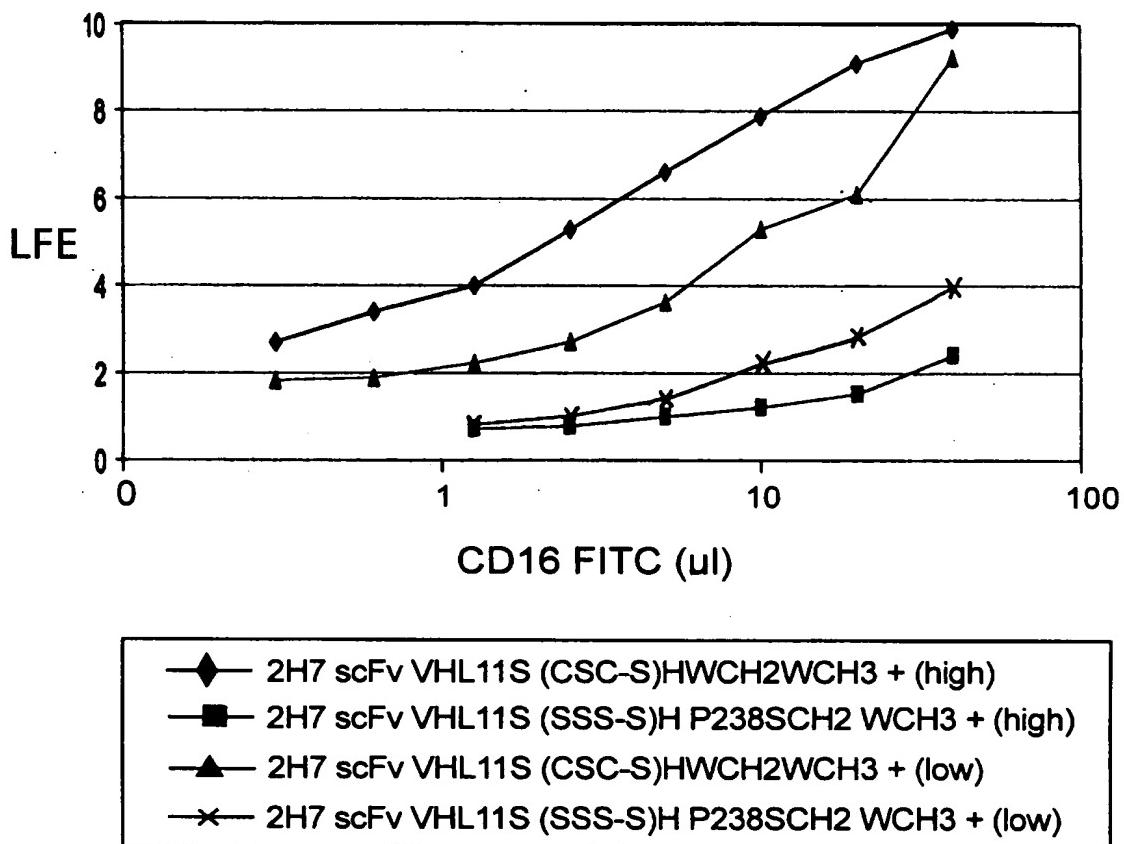
**FIG. 68**

**CD16(ED)(SSS-S)H P238S CH2 WCH3 high and low affinity alleles expressed as soluble molecules**



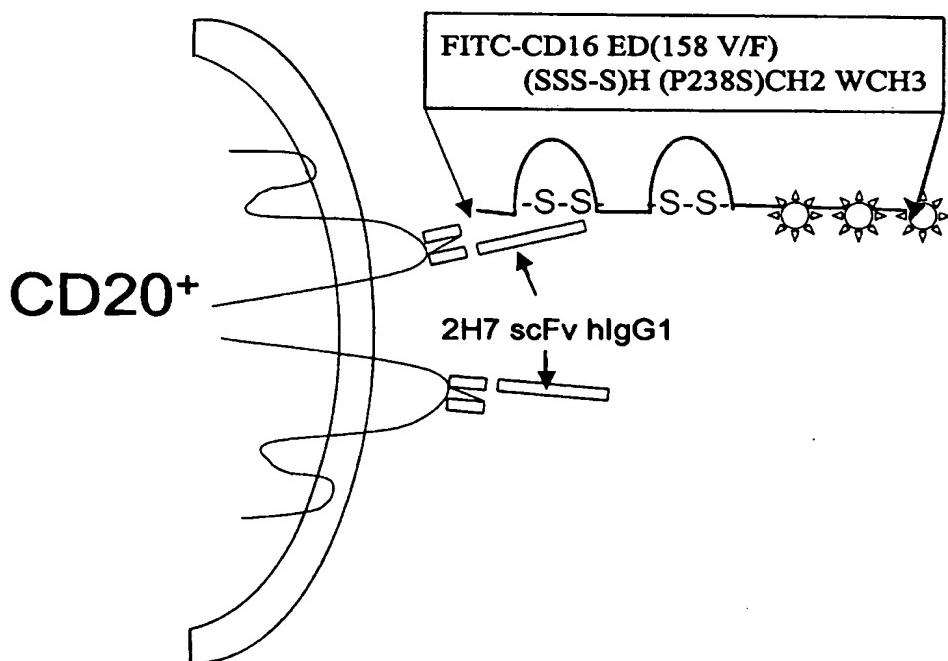
**FIG. 69**

**Binding of soluble CD16-FITC high and low affinity fusion proteins  
to 2H7 scFv VHL11S (CSC-S)H WCH2WCH3 or  
(SSS-S)H P238S CH2WCH3 on CD20CHO Targets**



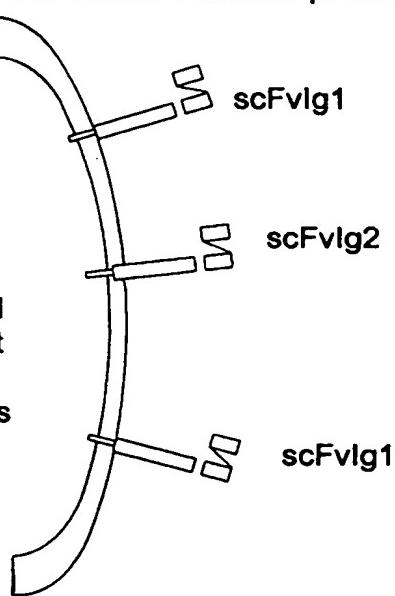
**FIG. 70**

**Binding of FITC Labeled, Recombinant Human CD16(ED) extracellular domain -Ig Fusion Protein to CytoxB Derivatives on CD20 CHO Cells**



**Expression of surface displayed SMIPs links modified cDNAs with the altered fusion proteins**

**Mammalian Cell Transfected With**  
1.A single surface displayed scFvlg expression construct  
OR  
2.a library of such molecules



**FIG. 71****CD37 mAbs and scFvIg Induce Apoptosis**

Bjab Staining	Annexin V Positive	
No scFvIg	17.5	
2H7 MH	27	
G28-1 MH	30.6	
G28-1 IgAH	28.9	
HD37 MH	29.1	
(2H7+G28-1)MH	41	
(2H7+HD37) MH	37.1	
(G28-1+HD37) MH	35.3	
		plus GAM
Ramos	AnnexinV Positive	AnnexinV positive
cells alone	3	3.3
2H7 Mab	1.4	3.1
G28-1 Mab	18.3	8.7
mAbs	HD37 Mab	3.7
	G28-5	3.9
	2H7+G28-1	32.3
	2H7+HD37	5
	2H7+G28-5	5.7
	HD37+G28-1	26.9
	HD37+G28-5	8.2
	G28-1+G28-5	39.5
		68.3